16 November 2007 | S10

Science

ROBOTICS

AAAS

Volume 318, Issue 5853

COVER

A collection of undersea robots and ocean-observing platforms monitor upwelling during the 2003 Autonomous Ocean Sampling Network program in Monterey Bay, California. See the special section on robotics beginning on page 1083.

Image: David Fierstein

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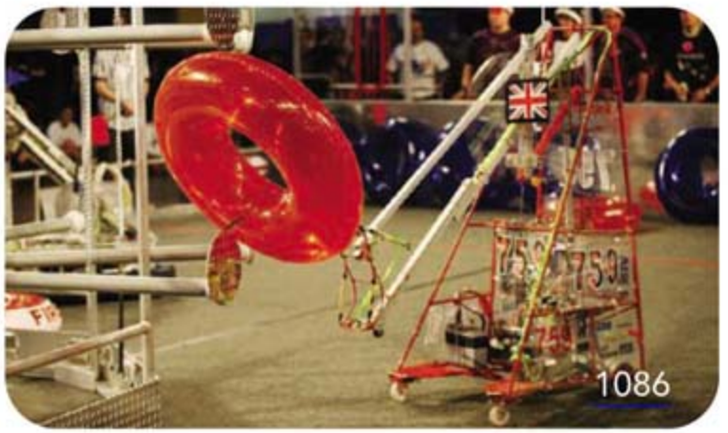
Editorial p. 1037; News stories pp. 1055, 1056, and 1060; Report p. 1155; for online material, see p. 1031 or go to www.sciencemag.org/sciext/robotics/



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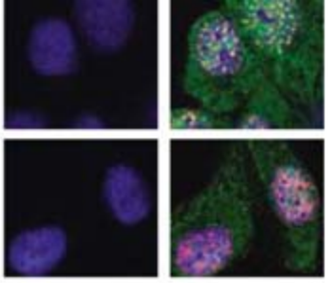

SCIENCE EXPRESS

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10.1126/science.1150034

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10.1126/science.1150179

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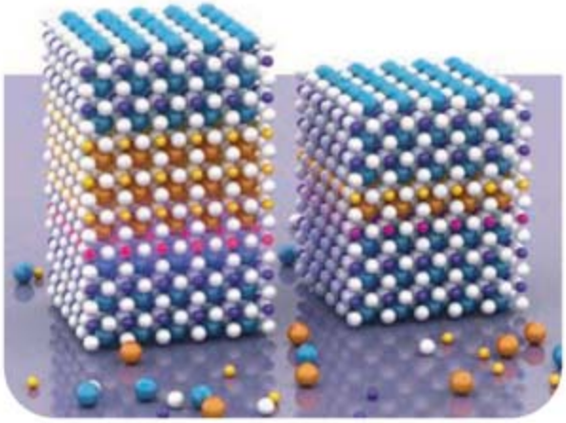

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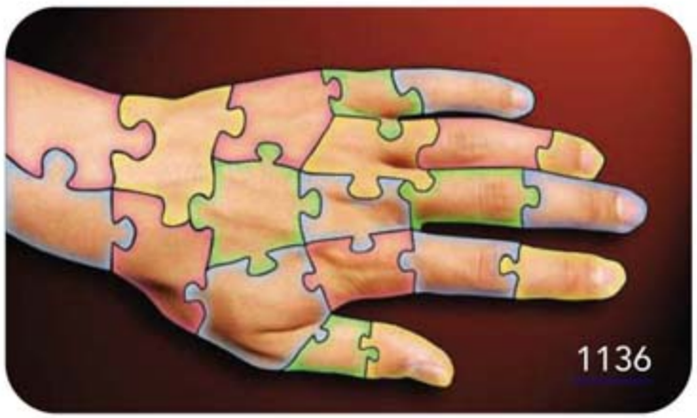

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Dental clues from an ancient ape.

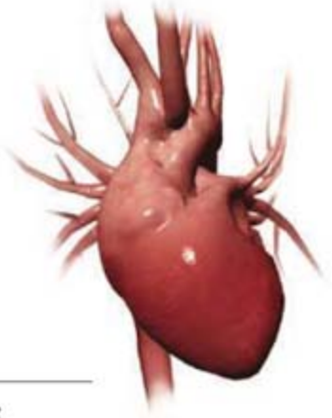
SCIENCENOW

www.sciencenow.org DAILY NEWS COVERAGE

Superbug Knocks Out Patient's Defense
Strains of MRSA found outside of hospitals destroy white blood cells.

New Ape Fossils Found in Africa
Discovery casts doubt on notion that apes went extinct in Africa and were replaced by others from Eurasia.

Glia Stoke Morphine's Fires
Helper cells in the brain are linked to addictive effects of opioids.



Small hearts and long life.

SCIENCE'S STKE

www.stke.org SIGNAL TRANSDUCTION KNOWLEDGE ENVIRONMENT

PERSPECTIVE: Adenylyl Cyclase 5—A New Clue in the Search for the "Fountain of Youth"?
J. A. Chester and V. J. Watts
A deficiency in adenylyl cyclase type 5 leads to a long life span.

PERSPECTIVE: Entrainment of the *Drosophila* Circadian Clock—More Heat Than Light
J.-Y. Fan, M. J. Muskus, J. L. Price
A study now provides the first insight into the molecular mechanisms by which temperature cycles synchronize circadian rhythms with the environment.

Finding qualities beneath the surface.

SCIENCE CAREERS

www.sciencecareers.org CAREER RESOURCES FOR SCIENTISTS

US: In Person—Hiring for Hidden Value
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A technical professional describes innovative ways of uncovering hidden talent.

US: Opportunities—The Golden Chapter
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Create your own career options by considering the direction you want to head in and then seeking opportunities to get you there.

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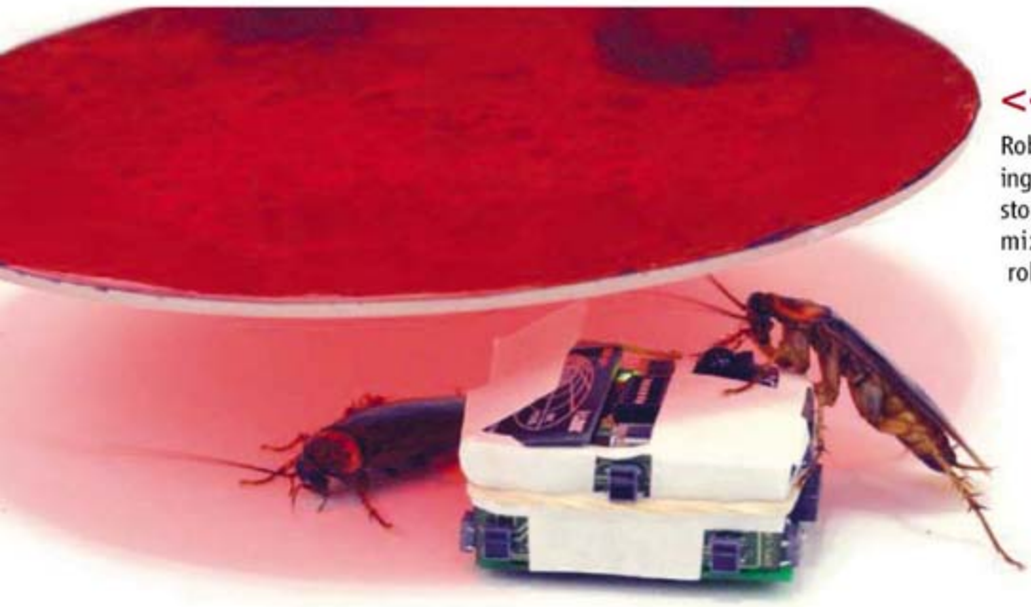
US: From the Archives—Supply Without Demand
D. Kennedy, C. Taylor, K. Urquhart, J. Austin
Bad estimates have become routine in the part of science policy called "scientific manpower."

SCIENCEPODCAST

Download the 16 November *Science* Podcast to hear interviews relating to this week's special section on robotics. Topics include robotics in extreme environments, robot ethics, the social behavior of robot cockroaches, and more.
www.sciencemag.org/about/podcast.dtl

Separate individual or institutional subscriptions to these products may be required for full-text access.



<< Cockroach Coercion

Robotics offer new possibilities for studying and modulating animal behavior. **Halloy *et al.*** (p. 1155; see the news story by **Pennisi**) observed collective decision-making by mixed groups of cockroaches and autonomous mini-robots. The robots, similar in size (though not in shape) to the cockroaches, were coated in a blend of cuticular hydrocarbons that mimic the natural cockroach cuticle. The robots and the insects made shared decisions regarding choice of shelter, and the robots could modulate the collective decision-making process and produce a behavior pattern—choice of an inappropriate shelter—not observed in groups of cockroaches alone. Thus, a small number of robots can change the global pattern by altering feedbacks between individuals in the system.

Reconstructing Interface Orbitals

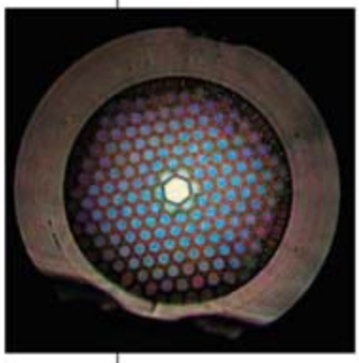
The rich phase diagram of the transition metal oxides and the recent demonstrations of patterning and tuning the interface region, which can lead to unexpected phenomena such as the quantum Hall effect and superconductivity, have generated much interest in developing oxide electronics. **Chakhalian *et al.*** (p. 1114, published online 11 October; see the Perspective by **Dagotto**) now report on an x-ray spectroscopy study on the interface between high-temperature superconducting $(Y,Ca)Ba_2Cu_3O_7$ and metallic $La_{0.66}Ca_{0.33}MnO_3$ with surface-sensitive as well as bulk-sensitive configurations. Charge transfers from the Mn oxide layer to the Cu oxide layer, but does not simply move into the $d_{z^2-x^2}$ orbitals of Cu in a rigid manner. Instead, orbital reconstruction occurs that involves population of the $3_{z^2-r^2}$ orbital. Calculations show that the orbital reconstruction is consistent with a scenario in which the Cu atom forms a covalent bond with the Mn atom.

Entropy-Driven DNA Networks

A key aspect of electronic circuits is amplification or gain, so that low signals can be distinguished from any persistent background. **Zhang *et al.*** (p. 1121; see the Perspective by **Bar-Ziv**) show how gain can be achieved in biochemical circuits. They have designed complex catalytic networks based on DNA in which the output oligonucleotides that are released go on to act as catalysts for other reactions. The process is designed to be entropy driven so that the pathways for reactions are well controlled and can be modified at will. Possible applications lie in the field of catalysis, sensor development, the development of enzyme-free alternative for the polymerase chain reaction, and the construction of nanomachines.

Photonic Route to the High Notes

Excitation of microstructured optic fibers with intense femtosecond laser pulses can generate broadband white light, and applications range from metrology to the generation of ultrafast pulses of a chosen wavelength. The principles behind white-light generation and optical guidance have not been well understood, and the control of the location and spectral range of the generated light has been limited. **Couny *et al.*** (p. 1118) developed theoretical insights into the guidance of light and generation of higher-order modes in the microstructured fibers that has allowed the generation of a frequency comb spanning an extremely wide spectral range with modest input power. The authors discuss how this approach could enable a simplified route to femto- and attosecond pulse generation and arbitrary waveform synthesis.



CREDITS (TOP TO BOTTOM): ULB AND EPFL; FETAH BENABID/UNIVERSITY OF BATH

Equatorial Water on Mars?

Water on Mars is primarily locked up in the polar ice deposits. **Watters *et al.*** (p. 1125, published online 1 November; see the Perspective by **Schultz**) show that comparable amounts of water could be hidden at the equator in the hills of the Medusae Fossae Formation, which are believed to be formed of volcanic ash and wind-blown sediments. Radar sounding with the Mars Advanced Radar for Subsurface and Ionospheric Sounding instrument aboard the Mars Express spacecraft saw reflections from the underlying terrain beneath the sediments that have a dielectric signature consistent with the presence of water ice. If these hills are ice rich, they must contain more dust and sand than the polar layered deposits but could host a volume of water similar to that of the south polar layered deposits.

Megasplay Faults and Tsunami Production

Tsunamis often result when there is rapid uplift of the sea bed, but their size is very sensitive to the local fault geometry. Megasplay faults, which are long thrust faults that rise from the plate boundary megathrust and intersect the sea floor, are thought to be particularly effective in transferring displacement to the surface. By creating a three-dimensional seismic view of a megasplay fault zone in the Nankai Trough off Japan, **Moore *et al.*** (p. 1128) show how such a fault operates and suggest that its slip may have

Continued on page 1035

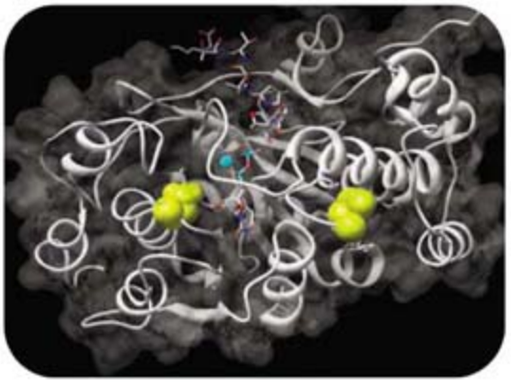
Continued from page 1033

contributed to devastating historic tsunamis such as the 1944 Tonankai event. Similarly, megasplay geometries may affect tsunami generation in subduction zones worldwide.

Spreading the Silence

The maternal and paternal copies of most genes are generally thought to be expressed at comparable levels, but there are several examples known where this is not the case. Imprinted genes have either the maternal or the paternal allele shut down, and in X-inactivation, one of the two X chromosomes is silenced. A few small classes of genes—including immunoglobulins, T cell receptors, and interleukins—are known to have one or other copy inactivated. **Gimelbrant *et al.*** (p. 1136; see the Perspective by **Ohlsson**) looked across the entire human genome and found that in 5% of analyzed loci either the paternal or the maternal allele is randomly and stably inactivated. This fraction is much higher than had been anticipated.

Cancer's Mutational Landscape



The genomes of human tumors contain many sequence alterations, a subset of which help drive tumor growth. **Wood *et al.*** (p. 1108, published online 11 October; see Perspective by **Trent and Touchman**) have now undertaken a systematic sequence analysis of >18,000 genes in human breast and colorectal tumors. Depiction of the mutational data on a topographic map indicates that each of these tumor types contains only a few gene "mountains" mutated at high frequency and a much larger number of gene "hills" mutated at low frequency. Importantly, while a large fraction of the mutations driving tumor growth reside in the gene hills rather than the mountains—a finding that underscores the heterogeneity of human cancer—it appears that many of the mutated genes function through cellular signaling pathways that are already well known.

The Yin-Yang of Inflammatory Responses

Inflammatory responses in the nervous system are very tightly regulated. In particular, T helper 1 type T cell responses must be kept in check, and a potent negative regulator of these cells is the surface receptor TIM3, a member of the T cell immunoglobulin and mucin family. However, **Anderson *et al.*** (p. 1141) report the unexpected finding that TIM3 also promotes inflammation through expression on cells of the innate immune system—namely, dendritic cells and microglia of the brain. The opposing roles for the same immune protein when expressed on different populations of immune cells raises intriguing questions about the balance between the promotion and inhibition of tissue inflammation.

Playback During Sleep

During sleep, hippocampal cells play back sequences of activity recorded beforehand during running of a maze task. **Euston *et al.*** (p. 1147) report a similar, although more compressed, form of activity playback, but in the medial prefrontal cortex (mPFC) rather than the hippocampus. The mPFC has been implicated in memory storage and retrieval, and it receives direct inputs from hippocampus.

Spinal Cord Injury and Cortical Compensation

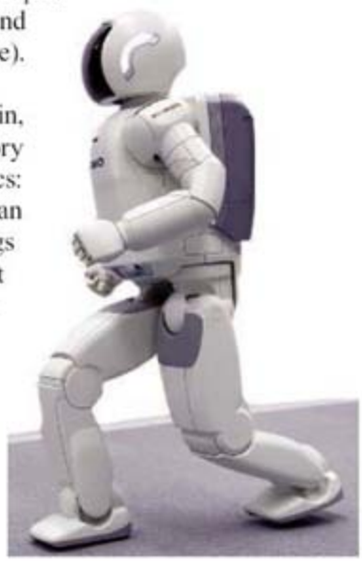
Neuro-rehabilitation is based on the concept that training recruits intact neuronal systems to compensate for brain injury. However, the neuronal basis of the underlying mechanisms is still poorly understood. **Nishimura *et al.*** (p. 1150) carried out a longitudinal study in macaques using a well-defined lesion of the direct cortico-motoneuronal connection at mid-cervical segments of the spinal cord. Functional recovery after lesion of the corticospinal tract involved a variety of widely distributed cortical networks. The contribution of each different cortical region changed depending on the post-operative recovery stage.

CREDIT: WOOD *ET AL.*

Robert J. Sawyer is a Hugo and Nebula Award–winning science-fiction writer. E-mail: sawyer@sfwriter.com

Robot Ethics

C-3PO AND R2-D2 MAY BE TWO OF THE WORLD'S MOST FAMOUS FICTIONAL ROBOTS, but a quasi-robot named MQ-5B/C is perhaps more interesting just now. On 1 September 2007, operators used this unmanned airborne drone to locate and drop a bomb on two individuals who appeared to be planting explosives near Qayyarah, Iraq. As we make robots more intelligent and autonomous, and eventually endow them with the independent capability to kill people, surely we need to consider how to govern their behavior and how much freedom to accord them—so-called roboethics. Science fiction dealt with this prospect decades ago; governments are wrestling with it today. Why now? It's not only because robots are killing people. It's also because they have become household consumer-electronics items and because some now look and act like humans (Honda's Asimo can even dance). We have an instinctive reaction that a threshold has been crossed.



The notion of killer robots is a mainstay of science fiction; but then again, so is the idea of robots with built-in safeguards against that. In his 1942 story "Runaround," Isaac Asimov offered his now-famous Three Laws of Robotics: A robot may not injure a human being or, through inaction, allow a human being to come to harm; a robot must obey orders given to it by human beings except where such orders would conflict with the First Law; and a robot must protect its own existence as long as such protection does not conflict with the First or Second Law. Most of Asimov's stories deal with things going awry because these laws don't equip robots to tackle real-world situations. In his 1947 story "With Folded Hands," Jack Williamson had robots adhere to an even simpler directive: To serve and obey, and guard men from harm. That, too, had an unwelcome result: a totalitarian society in which robots prohibit humans from participating in almost all activities, lest one of us be injured.

Indeed, all attempts to govern complex robotic behavior with coded strictures may be misguided. Although the machines will execute whatever logic we program them with, the real-world results may not always be what we want. And yet, we seem unable to resist trying, and so governments are now drafting their versions of Asimov's and Williamson's laws. This year, South Korea's Ministry of Commerce, Industry, and Energy established a Robot Ethics Charter, which sets ethical guidelines concerning robot functions. The move anticipates a time when intelligent service robots are part of daily life. EURON (the European Robotics Research Network) also announced plans to develop guidelines for robots in five areas: safety, security, privacy, traceability, and identifiability. Japan's Ministry of Economy, Trade, and Industry has joined in too. With an aging population and robot caregivers being developed there (and elsewhere in the world), the Japanese foresee robots in many homes and have issued policies for how they should behave and be treated.

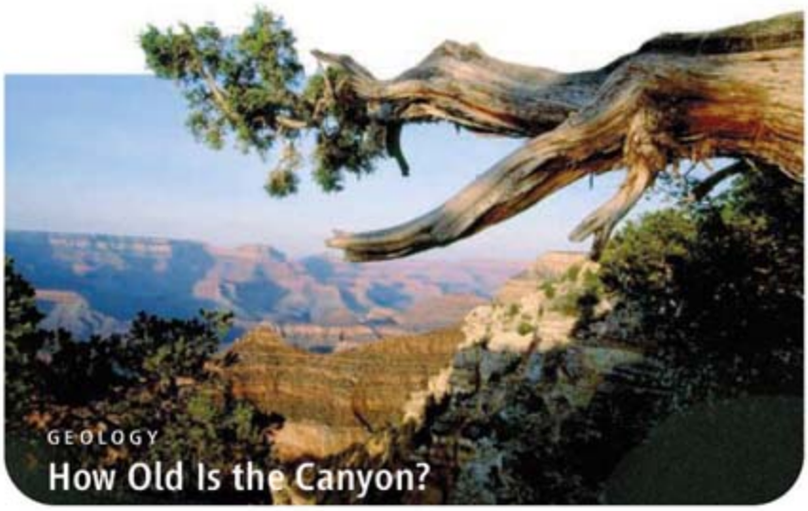
The United States has yet to jump on the roboethics bandwagon. That many U.S. robots are created for the military and designed to harm humans may be the reason. Still, it is likely that the most interesting litigation defining robot responsibilities and rights will emerge in the United States. For starters, a Michigan jury awarded the family of the first human ever killed by a robot (accidentally, in 1979) $10 million, which was, at that time, the largest personal-injury award in the state's history.

Again, science fiction may be our guide as we sort out what laws, if any, to impose on robots and as we explore whether biological and artificial beings can share this world as equals. Isaac Asimov's 1954 novel *The Caves of Steel* describes a fully equal robotic partner of a police officer. Lester del Rey's 1938 story "Helen O'Loy" portrays what might be one viable future: a man marrying a robot woman, and living, as one day all humans and robots might, happily ever after. I, for one, look forward to that time.

– Robert J. Sawyer

10.1126/science.1151606

CREDIT: CAROLYN CLINK; HONDA



GEOLOGY

How Old Is the Canyon?

Much about the timing of formation of the Grand Canyon in Arizona remains uncertain. The process is closely tied into the history of uplift of Western North America during the Cenozoic, including even recently, as well as regional climate change. One approach toward improved understanding is to date past positions of the Colorado River as it deepened the canyon. To do this, Karlstrom *et al.* have taken advantage of the many volcanic fields in the western part of the canyon; some of these poured lava into the canyon during the past several million years. In fortunate cases, remnants of these flows are preserved perched on ledges or beaches in the canyon, marking past river levels. $^{40}Ar/^{39}Ar$ dating of these young flows shows that the western part of the canyon has continued to deepen by about 100 to 150 m since about 1.5 million years ago. To the east, across a major fault, the canyon has been deepening at 2 to 3 times that rate. Further dates show that this pattern of active differential uplift, facilitated by faulting, has operated over the past 5 to 6 million years and has continued to modify the canyon even geologically recently. — BH

Geol. Soc. Am. Bull. **119**, 1283 (2007).

MICROBIOLOGY

More than Skin Deep

Skin fungi cause conditions ranging from flaky scalps and eczema to weeping dermatitis and invasive disease. Major culprits are the *Malassezia* spp., which are closely related to plant pathogenic basidiomycetes, such as *Ustilago maydis*. In a proteomic-genomic study, Xu *et al.* discovered that when *Malassezia* grows on the human scalp, it secretes over 50 proteins, which are generally more similar to those secreted by other skin-parasitizing fungi, such as *Candida albicans*, than to those of its plant parasite cousins. The secreted proteins include allergens responsible for atopic eczema, but the ones critical for the *Malassezia* lifestyle are lipases; these enzymes are required in order to harvest host lipids in compensation for an apparent fungal inability to synthesize fatty acids de novo. The secreted enzymes include a distinctive arsenal of extracellular hydrolases, another similarity to *Candida*. Furthermore, sequencing of the haploid genome revealed mating type genes and a pheromone-responsive MAP kinase module, like those found in yeast. It could be that sex promotes skin colonization and the exchange of virulence determinants. — CA

Proc. Natl. Acad. Sci. U.S.A. **104**, 10.1073/pnas.0706756104 (2007).

MATERIALS SCIENCE

A Matter of Coexistence

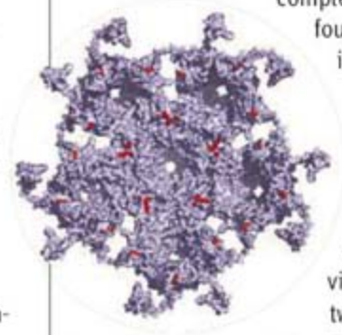
Alloys such as $Ge_2Sb_2Te_5$ (GST) find use in nonvolatile electronic memory and as recording media in DVDs because they undergo a fast and reversible transition between amorphous and crystalline phases with distinct optical and electronic properties. It was initially assumed that the amorphous form was simply a disordered version of the metastable cubic (rock salt) form. However, experiments on quenched thin films and simulations have given a different and often conflicting picture. Caravati *et al.* used ab initio molecular dynamics simulations to probe the amorphous structure. They started with metastable cubic GST with Te occupying one sublattice and Ge, Sb, or vacancies randomly occupying the other. For a quenched and annealed sample at 300 K, the calculated x-ray scattering factor was in good agreement with experimental results from the literature. The Ge and Sb atoms were mostly four-coordinate and the Te atoms mostly three-coordinate in defective octahedral-like sites, resembling cubic crystalline GST. However, about one-third of the Ge atoms occupied a tetrahedral environment, absent in the crystalline phase but supportive of the large number of homopolar Ge-Ge, Ge-Sb and Sb-Sb bonds that formed. The authors believe the coexistence of these two arrangements accounts for the rapid phase changes and strong optical contrast between the phases. — MSL

Appl. Phys. Lett. **91**, 171906 (2007).

VIROLOGY

Come In and Take Your Coat Off

The replication of animal viruses relies on their ability to cross a cellular membrane on their way into the host cell's cytoplasm. Simian virus 40 (SV40) is a non-enveloped DNA virus that enters cells via caveolar endocytosis, followed by vesicular transport to the endoplasmic reticulum (ER)–the entry portal of the host cell's secretory pathway–whence it crosses into the cytosol en route to the nucleus. Schelhaas *et al.* wondered why the viruses follow this relatively complex itinerary. They found that ER-localized enzymes that promote the isomerization of cysteines between their thiol and disulfide states were required for viral entry, and that two ER membrane proteins, Derlin-1 and Sel1L, which are known to mediate the retrotranslocation of misfolded host proteins from the ER back into the cytoplasm, were also important. Specifically, the oxidoreductase ERp57 catalyzed a rearrangement of disulfides within the capsid, resulting in a loosening of the pentamer-hexamer joints in the virus coat. Once in the cytosol, the reduced levels of calcium may promote viral capsid disassembly, facilitating release of the genome. — SMH



A portion of the capsid showing the target cysteines (red).

Cell **131**, 495 (2007).

Continued on page 1041

CREDITS (TOP TO BOTTOM): THEO ALLOFS/ZEFA/CORBIS; SCHELHAAS *ET AL.*, *CELL* **131**, 495 (2007)

Continued from page 1039

BIOCHEMISTRY

Back to Basics

Nitrogenase enzymes use an elaborate metal cluster to catalyze the reduction of dinitrogen to ammonia under remarkably mild conditions. Two questions about this process continue to puzzle researchers: What are the elementary steps underlying the scission of the nitrogen triple bond, and how is the cluster that guides these steps assembled? Curatti *et al.* shed light on the latter question by reconstituting from purified components a system for in vitro synthesis of the cluster—which contains 7 Fe, 9 S, Mo, homocitrate, and one as-yet unidentified light atom and is called the FeMo cofactor. Of 11 nitrogen fixation (Nif) proteins previously shown to be involved in FeMo cofactor biosynthesis, they find that NifB, NifEN, and NifH are key. NifB assembles ferrous iron, sulfide, and *S*-adenosylmethionine into the NifB cofactor (a precursor of the FeMo cofactor) under reducing conditions; NifEN pushes the synthesis one step further by converting the NifB cofactor into the VK cluster, to which molybdate and homocitrate are then added in a NifH-dependent fashion. The other Nif proteins are thought to supply the relevant forms of Fe, S, and Mo under in vivo conditions and also to protect labile intermediates. The catalytic competency of the synthesized FeMo cofactor (whose structure is still unknown) was confirmed by its ability to combine with apo-NifDK into a holoenzyme that reduced nitrogen. — GJC

Proc. Natl. Acad. Sci. U.S.A. **104**, 17626 (2007).

CHEMISTRY

Extending Networks

Many desirable materials properties tend to have tradeoffs. For example, engineered cross-linked polymer networks can have high tensile strength, but though lighter than metals, they have much poorer extensibility—their rigidity causes them to fail after a small increase in length. Some proteins, such as the muscle protein titin, do combine high strength and elasticity, in part because they have a modular structure that unfolds upon deformation. Kushner *et al.* mimicked this property in their design for cross-links in a poly(*n*-butyl acrylate) network. Side chains that could form four hydrogen bonds also carried long-chain terminal olefinic groups, which through ring-closing metathesis formed flexible covalent links between the hydrogen bonding pairs (much like the safety chain on a car trailer), providing two levels of chain cross-linking. Compared to a control material with a poly(ethylene glycol) cross-link, the tensile strength increased by 700% for similar elongations at a cross-link density of 6%. — PDS

J. Am. Chem. Soc. **129**, 10.1021/ja0742176 (2007).

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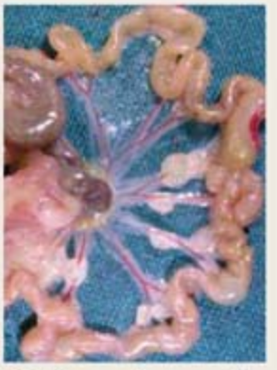
<< A New Angiogenesis Weapon



Metastases of a pancreatic tumor in mesenteric lymph nodes.

Tumors need blood, and they secrete angiogenic molecules such as vascular endothelial growth factor (VEGF) to encourage new blood vessels to form. Although an antibody directed against VEGF (αVEGF) can prolong life when given in conjunction with chemotherapy to individuals with certain cancers, inhibiting VEGF signaling can elicit adverse side effects and switch on alternative angiogenic mechanisms in tumor cells. Noting that placental growth factor (PlGF, a VEGF family member) is not required for normal development of the vasculature but has been implicated in pathological angiogenesis, Fischer *et al.* investigated the effect on tumors of an antibody directed against PlGF (αPlGF). In a mouse model, αPlGF by itself inhibited the growth or metastasis of melanoma and of colon and pancreatic carcinomas, and enhanced inhibition of tumor growth by the chemotherapeutic agents gemcitabine and cyclophosphamide, as well as the anticancer effects of an antibody directed against the VEGF receptor (αVEGFR). The processes inhibited included tumor angiogenesis and lymphangiogenesis, as well as the recruitment of proangiogenic macrophages. On the other hand, αPlGF did not turn on the expression of proangiogenic genes, nor did it mimic or enhance αVEGFR-dependent side effects; indeed, pregnant mice treated with αPlGF delivered litters of healthy pups. Thus, the authors hope that αPlGF might represent a useful addition to the anticancer armamentarium. — EMA

Cell **131**, 463 (2007).

CREDIT: FISCHER *ET AL.*, *CELL* **131**, 463 (2007)

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Training Your Gut

If you love chocolate, you can thank—or blame—the microorganisms in your gut. People who are hot for chocolate, researchers say, harbor different bacteria from those who are indifferent to the treat.

Biochemists from Nestlé chocolate company and Imperial College London spent 5 days studying 22 healthy volunteers, half of them avowed chocoholics, on a set diet. They analyzed urine and blood samples for the metabolic byproducts of different types of bacteria. The results, published in the November *Journal of Proteome Research*, reveal that levels of dozens of these compounds in the two groups differ.

The differences are "not just a product of our genes," says biochemist and project leader Sunil Kochhar, noting that Indians who move to the United States show changes in gut bacteria. Kochhar thinks they become attuned over time to a person's lifestyle and, in turn, can influence future preferences—sometimes in a positive direction. Researchers found that chocoholics' blood had lower levels of bad cholesterol and higher levels of albumin, a nutrient-carrying protein. Glenn Gibson, a microbiologist at the University of Reading, U.K., says understanding how diet affects gut activity can lead to the development of personalized nutrition plans that can nudge bacteria in the direction of good health.

Phony Scoop on CO_2

Global warming is actually caused by growing numbers of CO_2-emitting bacteria on the sea floor, says a study published online on 3 November in the *Journal of Geoclimatic Studies*. "Those who subscribe to the [human-caused climate change] theory have overlooked the primary source" of CO_2 emissions, write Daniel Klein and colleagues at the University of Arizona in Tucson.

The problem is that Klein and his team don't exist. Neither does their Department of Climatology; Okinawa University, where the journal is purportedly published; or its editor, OU climatologist Hiroko Takebe.

It's a hoax designed "to expose the credulity and scientific illiteracy of … 'climate skeptics,' " according to "Mark Cox," the self-described real author of the article. Cox says several anti–global warming Web sites cited the paper but hastily erased their coverage when the hoax was revealed. *Science* got an e-mail from Cox after speaking with David Thorpe, a U.K.-based science journalist and Web site designer. Thorpe says he created the site but denies writing the article.

The paper reports that algal blooms have gradually killed off "brachiopod molluscs of the genus *Tetrarhynchia*" and other organisms that prey on CO_2-producing bacteria, allowing bacteria populations to explode. The paper has "some clever ideas," says geochemist Steven D'Hondt of the University of Rhode Island in Kingston, but "some fairly fundamental flaws," such as meaningless equations. He also notes that brachiopods and mollusks are two different phyla.

ALL ABOARD FOR SCIENCE

Boys in Delhi pore over a globe that at the press of a button will show different peoples of the world speaking their native tongues. It's part of a science exhibit on a train that will travel 15,000 kilometers over the next 7 months, stopping in more than 55 cities throughout India. The 14-car-long Science Express is chock-full of multimedia exhibits covering topics such as black holes and CERN's search for the Higgs boson. It also has a hands-on lab where children can do experiments such as mixing cement or separating chemicals with paper chromatography. The $10 million Indo-German project is a mobile version of the Max Planck Society's long-running "Science Tunnel," which has been on display in Hannover, Germany, and has traveled to several cities in Asia.

A Probable Killer?

Is Lucia de Berk, a Dutch nurse, a serial killer or the victim of shoddy statistics? Dutch courts sentenced De Berk to life in prison for murdering seven patients and attempting to kill three others. But dozens of statisticians last week petitioned the Dutch justice department to reopen the case.

Suspicions first arose in 2001 after a 6-month-old girl died under murky circumstances while De Berk was on duty. Prosecutors found that nine other suspicious incidents had occurred during her shifts at three hospitals. Although no direct evidence implicated De Berk, the courts decided that it was unlikely—only one chance in 342 million, according to one witness—that so many deaths could have occurred accidentally while she was nearby.

That conclusion is based on "every statistical mistake in the book," says Leiden University statistician and petition organizer Richard Gill. For instance, he says, several deaths were deemed natural and only later declared suspicious by doctors who knew De Berk had been on duty. And fewer people died during De Berk's 2-year stint at one hospital ward than during the prior 2 years. "Nobody was murdered by anybody," Gill concludes.

De Berk shown as witchlike in cartoons.

Last month, a justice department panel recommended that the case be reopened. The decision now rests with the Supreme Court.

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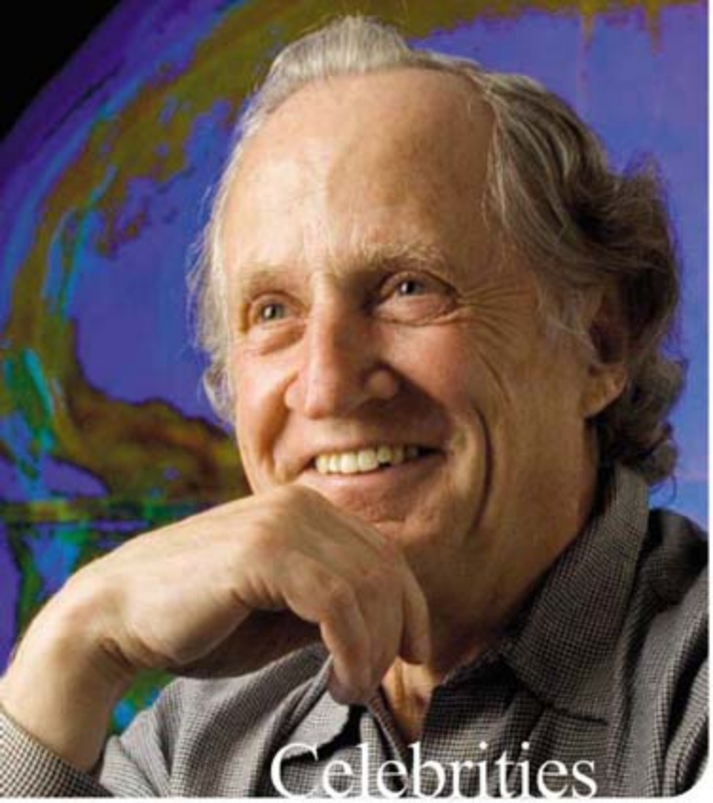

A SECOND LOOK. The gripping story of Mario Capecchi's childhood in wartime Italy made headlines throughout the world last month when he won the Nobel Prize in physiology or medicine (*Science*, 12 October, p. 178). But the extra fame has triggered a new level of scrutiny that casts doubt on some details.

The questions arose after the Associated Press (AP) reported that parts of the story Capecchi has repeatedly described—surviving on the streets from ages 4 to 9 after his mother was arrested in 1941 by the Gestapo and taken to the Dachau concentration camp—can't have happened that way. The AP found no record of Capecchi's mother being held at Dachau, and historians say that the Gestapo was not working in Italy then. Other records suggest that Capecchi spent at least part of that time living with his father. After AP showed him the new information, Capecchi released a statement saying that "what I have said and written is my most accurate recollection of my early childhood." He has said that the stories were based on what his mother and uncle told him after the war.

A longtime collaborator, molecular biologist Kirk Thomas of the University of Utah in Salt Lake City, says the details of Capecchi's story are unimportant. "If you asked me what makes him a great scientist, I think growing up on the streets of war-torn Italy probably has something to do with it," he says. Capecchi's optimism, capacity to focus, and ability to make tough decisions "are all survival skills," Thomas adds.

MOVERS

TROPICAL TRAINING. A fledgling medical school in Singapore has lured tropical disease specialist Duane Gubler away from the sun and sands of Hawaii to set up a research program in emerging infectious diseases. The school, established by Duke University and the National University of Singapore, opened this summer with the goal of strengthening graduate-level medical training in the city-state.

Gubler, an expert on dengue, now directs the Asia-Pacific Institute of Tropical Medicine and Infectious Diseases at the University of Hawaii, Manoa. He expects to assemble a team of 10 principal investigators to do basic, clinical, and translational research, as well as to train grad students and postdocs. He also hopes to knit new field labs into an emerging infectious diseases regional network. "Singapore is in an ideal position to provide regional leadership," Gubler says.

ON FIRMER GROUND. After 2 years of uncertainty, the leading journal of environmental health has a new editor in chief. Hugh Tilson, 61, a neurotoxicologist and administrator at the Environmental Protection Agency, will head *Environmental Health Perspectives* (*EHP*), published by the National Institute of Environmental Health Sciences (NIEHS) in Research Triangle Park, North Carolina. In 2005, then–NIEHS director David Schwartz proposed privatizing the open-access journal but later scuttled the plan after protests from scientists, environmentalists, and Congress.

Tilson fills a slot that's been empty for the past year. He says NIEHS will restore cuts to *EHP*'s budget and bring back some features. He hopes to expand the role of associate editors and publish more research on risk assessment. "We are confident that [Tilson] will provide stability and scientific rigor to the journal," says Jennifer Sass of the Natural Resources Defense Council in Washington, D.C.

MONEY MATTERS

FOR GREATER GOOD. David Heymann has spent a lifetime fighting the spread of infectious diseases, most recently teaming up with epidemiologists and health professionals around the world to stop SARS in its tracks. Those efforts earned the 61-year-old World Health Organization expert a $250,000 prize from the Heinz Family Foundation last month, which he's now donating to work on the global monitoring and prevention of infectious diseases.

The money will go to the Emory University Global Health Institute in Atlanta, Georgia, to support the training of young epidemiologists in developing countries. The initiative, to be coordinated through the International Association of National Public Health Institutes (IANPHI)—a nonprofit funded by the Bill and Melinda Gates Foundation—is "totally consistent with David's own dedication and passion in public health," says IANPHI president and Heymann's longtime friend Jeffrey Koplan, who describes Heymann as a "remarkably humble and self-effacing soul."

"How could I keep this award to myself when there are so many others working to protect the human condition," Heymann says, citing individuals such as "Red Cross workers fighting Ebola or Marburg disease in the Congo" and "community volunteers trudging to remote villages to stop polio in Afghanistan."

<< INSIDE GOVERNMENT

DOE SHAKEUP. Patricia Dehmer has become the top research manager within the Department of Energy's (DOE's) $4 billion Office of Science, filling the new position of deputy director of science programs under Raymond Orbach. Orbach, who is also undersecretary for science, promoted Dehmer last month from head of basic energy sciences to overseer of all six DOE research programs—which also encompasses high-energy physics, nuclear physics, fusion, biological and environmental sciences, and advanced computing—whose managers previously reported directly to Orbach.

"Pat is an extremely effective communicator," says Michael Lubell of the American Physical Society. "She understands very, very well how to deal with members of Congress and with DOE."

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AIDS RESEARCH

Did Merck's Failed HIV Vaccine Cause Harm?

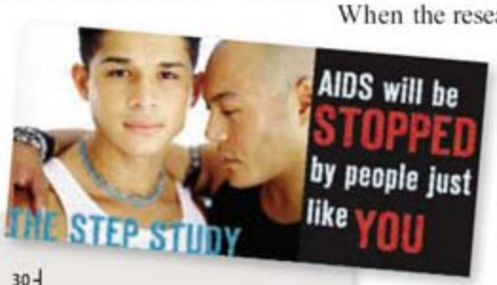
SEATTLE, WASHINGTON—A common cold virus has walloped the already ailing AIDS vaccine field.

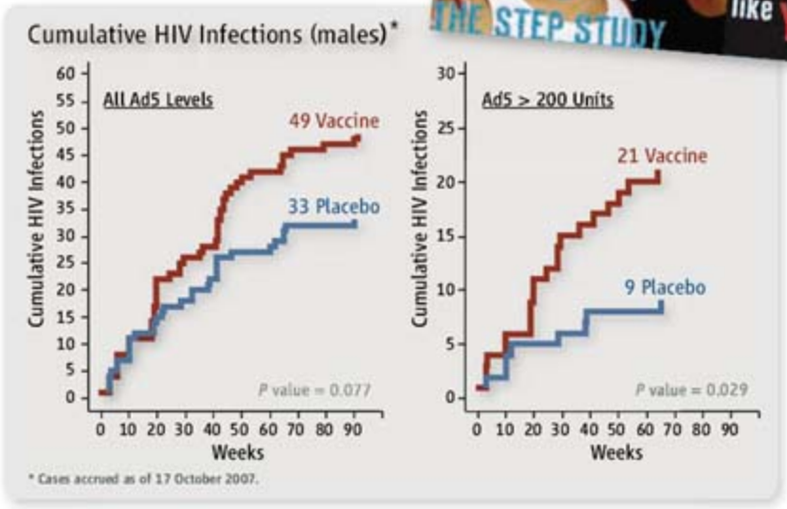
AIDS researchers, who are still staggering from the unexpected failure in September of the most promising vaccine candidate in clinical trials, met here last week to explore an even more alarming finding: The vaccine, made by Merck and Co., may actually have increased the risk of HIV infection in some study participants.

Working with the academic-based HIV Vaccine Trials Network (HVTN) and the U.S. National Institutes of Health (NIH) in Bethesda, Maryland, Merck researchers stopped the multicountry study after an interim analysis revealed that the vaccine did not work (*Science*, 5 October, p. 28). Now further analysis suggests that the vaccine may have helped HIV infect a subset of participants who at the trial's start had high levels of antibody to adenovirus 5 (Ad5), which causes the common cold and is also a component of the vaccine. "This is the worst possible outcome in a vaccine trial," said AIDS researcher Eric Hunter of Emory University in Atlanta, Georgia, one of the study sites.

The finding is as befuddling as it is frightening, and its implications are far-reaching. The data presented here to some 500 attendees at an HVTN meeting on 7 November found only a "trend" toward what's called "enhancement," leaving investigators wondering whether the elevated number of infections in vaccinees who had high Ad5 immunity was due to chance, behavior, or a vaccine-induced problem. Despite intensive investigations, no biological mechanism has emerged to explain how preexisting immunity to Ad5 could make vaccinated people more susceptible to HIV. "The data are very complex, and trying to understand what they mean has required an enormous amount of work," said Merck's Michael Robertson, a co-chair of the study.

In the first full accounting of the trial results, Merck researchers and their partners reported that, as of 17 October, HIV had infected 83 people in the placebo-controlled trial. Of these, 49 were vaccinated and 34 received saltwater injections. This difference clearly indicates that the vaccine does not protect against HIV, but the increased infections in vaccinees have no statistical import and likely are due to chance.





Double trouble. The vaccine clearly failed (*left*), but in men with high Ad5 antibodies (*right*), it may have increased their risk of infection. (Women were excluded from this analysis because only one became infected during the study.)

The discovery of possible enhancement in the so-called Step Study also owes something to chance. The vaccine contains three HIV genes stitched into a modified Ad5 vector that infects cells, creating HIV proteins that teach the immune system how to attack the real AIDS virus. From the outset, investigators worried that high levels of preexisting Ad5 antibodies might attack the vector and cripple the vaccine. So when Step began in December 2004, they enrolled 1500 people at high risk of becoming infected with HIV who had low Ad5 antibody levels. When data then suggested that this concern had been overblown, they doubled the trial size in July 2005 to include people with high Ad5 immunity. Most participants were men who have sex with men, although 38% were women, many of whom were sex workers.

The interim analysis in September that revealed the vaccine wasn't working looked only at the low–Ad5-antibody group. When the researchers subsequently examined the high–Ad5-antibody group, they were startled to find 21 infections in vaccinees versus nine in the placebo group.

The statistical analysis is ambiguous. Typically, researchers deem a difference as significant if it has a 95% probability of not being due to chance—a P value of less than 0.05. By these standards, the finding, with a P value of 0.029, was significant. But Steven Self, HVTN's head statistician at the University of Washington (UW), Seattle, cautioned that this comparison merits a more stringent cutoff for significance, between 0.025 and 0.0025, because the study was not designed to assess potential harm, nor did investigators plan to evaluate a subset of the study population. Still, Self said this "trend" deserves close examination.

Several researchers described their recent efforts to make sense of the trial's results. UW's Juliana McElrath, an immunologist who directs HVTN's lab program, explored what many consider the most likely explanation: that people in the high–Ad5-antibody group were more vulnerable to HIV because of "immune activation." Specifically, HIV establishes an infection by attaching to T cells that have surface receptors known as CD4 and CCR5. Natural infection with Ad5 creates memory banks of these very T cells, which expand and direct an attack if Ad5 shows up again. Theoretically, the vaccine vector could have activated these memory cells in the ▶

SOURCE: MERCK; (INSET) THE STEP STUDY, HVTN

An ocean of undersea data
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same way, creating more targets for HIV. But McElrath's preliminary work found no evidence for this scenario.

Behavioral changes don't seem to provide an explanation: Study co-chair Susan Buchbinder of the San Francisco Department of Public Health said risk behaviors had decreased across the board and more so in the high–Ad5-antibody group. Buchbinder said investigators still are sorting out many variables related to HIV transmission, including circumcision, coinfection with other sexually transmitted diseases, and genetic factors.

One thing is clear: The monkey studies that suggested that the vaccine could thwart the AIDS virus, fueling much excitement, misled Merck researchers. "Mice lie, monkey sometimes lie, and humans never lie," said Peggy Johnston, head of NIH's AIDS vaccine program. "Some monkeys have lied to us this time." Other attendees stressed that Merck relied on a wimpy strain of the AIDS virus to "challenge" vaccinated monkeys and that challenges with stronger strains predicted that the vaccine would fail.

Although the mechanism remains elusive, researchers struggled with whether to tell trial participants if they received the vaccine or the placebo. A more recently launched study of the same vaccine in South Africa was stopped and quickly "unblinded" after learning the Step results, notifying everyone of their vaccine status (*Science*, 2 November, p. 729). After much debate here, Step's scientific steering committee recommended unblinding, and an oversight committee concurred on 13 November.

The specter of enhancement also affects the AIDS vaccine field's next-best hope. This NIH-made vaccine uses a similar Ad5 vector and was slated to enter a $130 million trial this fall without screening people for Ad5 immunity. "Step's results demand that we reexamine and redesign our study," said principal investigator and Step collaborator Scott Hammer of Columbia University.

Merck's Mark Feinberg warned colleagues that "the whole field will come apart at the seams" if it doesn't properly investigate and respond to the Step results. "I've never seen more complicated data to emerge from a study," Feinberg said. "And this one focuses on as important a question as I've ever known."

–JON COHEN

EPIDEMIOLOGY

Privacy Policies Take a Toll on Research, Survey Finds

A federal rule aimed at protecting patient data is hindering epidemiology research, adding costs and delays without enhancing confidentiality, according to a study this week in the *Journal of the American Medical Association* (*JAMA*). The survey responses from 1500 epidemiologists reflect the first systematic analysis of privacy rules that researchers have complained about for 4 years.

The problems stem from the Health Insurance Portability and Accountability Act (HIPAA), passed by Congress 11 years ago to make it easier for people to transfer their health insurance. A so-called Privacy Rule that took effect in April 2003 requiring health care providers to protect the privacy of medical records also affects research. Investigators must get permission to use a patient's medical data, even to identify potential participants. If that is not possible, the researchers can try to get by with a data set stripped of identifiers, such as name and address, or they can seek a waiver from an institutional ethics board.

These requirements have had a major impact on population-based health research, according to the survey, headed by epidemiologist Roberta Ness of the University of Pittsburgh in Pennsylvania. Survey invitations were e-mailed to more than 10,000 members of 13 epidemiology societies, and 1537 of them completed a Web survey. About 68% said the Privacy Rule has made research a great deal more difficult; half reported major delays; and nearly 40% faced much higher costs (see table). Only one-quarter said the rule has greatly improved confidentiality. Of those who modified a protocol to comply with HIPAA, two-thirds said it was much harder to recruit subjects.

Epidemiologists' Views on the Privacy Rule

	None	Some	A great deal
Made research more difficult	9%	16%	68%
Enhanced confidentiality	47%	20%	26%
Added cost	22%	21%	39%
Delayed time to study completion	21%	19%	51%

Note: Based on 1527 responses.
Results total less than 100% because they do not include responses of "don't know."

SOURCE: R. NESS, *JAMA* 298, 18 (14 NOVEMBER 2007)

Overprotected? A rule meant to ensure the privacy of medical data is hampering research, according to survey of epidemiologists.

The results support anecdotal evidence that the Privacy Rule has slowed enrollment and threatened some studies, says Ness (*Science*, 9 July 2004, p. 168; 17 March 2006, p. 1547). For example, at the University of Michigan, researchers were required to obtain consent for a survey of patients with heart disease care by mail rather than by phone, resulting in a drop in the response rate from 96% to 34% and a bias toward older, healthier, married participants. Ness's survey also suggests that U.S. surveillance of infectious diseases may be suffering because hospitals aren't sure what they can report.

Three years ago, an advisory panel urged the Department of Health and Human Services (HHS), which administers the Privacy Rule, to ease the burden on researchers by revamping the rule. The agency never formally responded. But HHS and other organizations commissioned the U.S. National Academies' Institute of Medicine (IOM) to examine the issue broadly; one of the results is the *JAMA* survey. Researchers in other disciplines have told the panel of difficulties, too. For instance, clinical oncologist Richard Schilsky of the University of Chicago Medical Center says HIPAA has been "a huge problem" for studies involving tissue samples, among others. Ness says she and her colleagues "really are hoping" that the IOM panel will devise recommendations that produce action. Its report is due by early 2009.

–JOCELYN KAISER

ENVIRONMENT

Panel Calls for Pilot Program For National Indicators

U.S. agencies that track the health of the environment should kick-start a pilot project to establish a national system of environmental indicators, a blue-ribbon review panel has recommended. In the same way that the gross national product tracks the state of the economy, environmental indicators, such as the area of wetlands, would monitor progress toward meeting environmental goals. The panel urged the agencies to start now and develop a plan for the remainder of the Bush Administration; they recommended water quantity stored in lakes, aquifers, and snowpacks as a test-bed indicator. Observers last week voiced support for a pilot project but stressed that users and contributors of data must be included in the design process if it is to be politically viable.

Many agencies monitor aspects of the environment. In 2004, the Government Accountability Office recommended that the White House Council on Environmental Quality (CEQ) figure out how to coordinate federal efforts. After a yearlong series of meetings with states and nongovernmental organizations, CEQ in 2006 asked an interagency team to figure out how to proceed. The team's white paper, finalized in September, laid out several options and recommended the creation of an interagency council to set policy, another interagency team to manage the technical work, and an advisory panel. The Department of the Interior (DOI), one of five agencies involved, had asked the National Academy of Public Administration to evaluate the team's ideas.

Chaired by Hermann Habermann, former chief statistician of the U.S. Census Bureau, the panel last month sent DOI an advance summary of its final report* due out in December. The panel agreed with the overall approach but stressed the need for immediate action. "What's needed at this juncture is not a new organizational chart but concerted leadership," said project manager Don Ryan at a workshop held last week by the nonprofit National Council for Science and the Environment.

A national indicator of water quantity would be a good place to start, Ryan explained, because this measure is relatively straightforward—compared to water quality, for example—and people care about supplies of water for drinking, irrigation, and wildlife. Ultimately, dozens of indicators might cover everything from air quality to biodiversity to outdoor recreation. The panel felt that the agencies should request funds for the pilot project in the 2009 budget; although it didn't come up with a figure, Ryan says "it's not a huge sum to get rolling."

CREDIT: JOHN BAZEMORE/AP PHOTO

Shrinking reservoir. Water scarcity, such as Atlanta is experiencing, could be a prototype national indicator of the state of the environment.

Reaction was mixed. Although the panel recommended that indicators be useful to high-level policymakers, such a "crosscutting indicator of water availability for the whole country doesn't make much sense," said CEQ's Ted Heintz at the workshop. "Water use is local." However, he and others emphasized that the main point of a pilot program would be to help federal agencies learn to work together better, and for that, water quantity might suffice.

Robin O'Malley, who heads the Heinz Center's Environmental Reporting Program in Washington, D.C., and others say that building broad support for a national system of indicators will be especially crucial now, because the political leadership at the agencies will change with the new Administration in January 2009. "The action plan has got to be about making sure it's rooted in the community when there is no one at the transition to save this," O'Malley said. "You take a big risk by keeping people out," he says.

Officials at DOI and CEQ are expected to decide in the next few weeks about whether and how to proceed. **–ERIK STOKSTAD**

* *A Green Compass: Institutional Options for Developing a National System of Environmental Indicators.*

Texas Votes for Cancer Research

Texas researchers are looking ahead to 2009 for the first grants from a $3 billion bond measure that voters approved easily last week. The project, which will fund as much as $300 million a year for 10 years in cancer research, was championed by several prominent Texans, including Governor Rick Perry and Lance Armstrong, the former cycling champion and cancer survivor (*Science*, 31 August, p. 1154). John Mendelsohn of the University of Texas M. D. Anderson Cancer Center in Houston says he expects that "outstanding scientists, hopefully from outside the state," will help review research proposals for the institute. **–JOCELYN KAISER**

Tests on Tests Urged

A group of scientific advisers wants the U.S. Department of Health and Human Services to tighten oversight of genetic tests, a growing enterprise regulated by a patchwork of federal rules. Last week, the Secretary's Advisory Committee on Genetics, Health, and Society released a 192-page draft report on genetic testing that called for new research to evaluate the clinical utility of genetic tests and for the expansion of public databases of gene mutations. The group also urged better proficiency testing of labs performing genetic tests.

"There's been very little movement forward" in more aggressively regulating these tests, says Gail Javitt of the Genetics and Public Policy Center in Washington, D.C., whose director helped write the report. Public comments are due by 21 December (www4.od.nih.gov/oba/SACGHS/public_comments.htm). **–JENNIFER COUZIN**

Europe Maintains Orbit

BERLIN—The European Space Agency (ESA) should maintain a human presence in low Earth orbit even if, as some have suggested, NASA pulls out of the international space station in the next decade, says ESA Director General Jean-Jacques Dordain. "I am convinced that utilization of the space station will bring scientific progress and technological progress," Dordain said at a press conference last week at the International Space Exploration Conference in Berlin. Although the space station without NASA "is a scenario that is very difficult to imagine," he said, "it will not be an end. We will continue to offer capability in low Earth orbit." ESA will unveil a formal proposal for the project next summer, Dordain says. Any decision about plans will come next November at a meeting of ministers from ESA member states. **–GRETCHEN VOGEL**

SCIENTIFIC WORK FORCE

New Analysis Questions Push for More Degrees

Academics, business leaders, and politicians have warned repeatedly that the United States risks losing its economic edge unless it produces more scientists and engineers. They also say that the country's system of science and math education is not up to snuff. But a new study* questions two basic tenets of that argument, concluding that work force data do not support claims of a looming labor shortage and that test scores indicate U.S. students are doing at least as well in science and math as their international counterparts are.

The supposedly sorry state of STEM (science, technology, engineering, and mathematics) education was a driving force behind enactment this summer of the America COMPETES Act, which authorizes $44 billion for a cornucopia of research and education programs across several federal agencies (*Science*, 10 August, p. 736). The bill drew heavily on a 2005 U.S. National Academies' report, the title of which, *Rising Above the Gathering Storm*, refers to the impending economic crisis facing the United States unless it bolsters STEM education (*Science*, 21 October 2005, p. 423).

But sociologist Harold Salzman of the Urban Institute and demographer B. Lindsay Lowell of Georgetown University, both in Washington, D.C., say that the academies' report paints a misleading picture and that its assumptions are leading to flawed STEM education policies. They note that the annual U.S. production of bachelor's, master's, and doctoral degrees in STEM fields has averaged three times the annual growth of science and engineering jobs between 1985 and 2000. They also point out that fewer than one-third of the 15.7 million workers with at least one STEM degree at any level hold jobs that require such training. Given those numbers, says Salzman, "expanding our production of scientists and engineers just defies market reality." Last week, Salzman made his case twice on the same day, at a talk at the Urban Institute titled "Houston, Do We Really Have a Problem Here?" and in a hearing before the House Committee on Science and Technology on how globalization affects the U.S. science and engineering work force.

The authors also say that U.S. students are learning more than critics give them credit for. For example, they note, math scores on the National Assessment of Educational Progress (NAEP) for students in eighth grade rose 15 points from 1973 to 2004. And contrary to popular belief that they trail the pack, says Salzman, U.S. students rank in the middle tier of countries on an international assessment of 15-year-olds in math and science.

Norman Augustine, former CEO of Lockheed Martin and chair of the panel that produced the *Gathering Storm* report, does not buy their arguments. In an e-mail to other members of the panel, Augustine notes that "what the [new analysis] does not observe is that an undergraduate degree in [science or] engineering is a prized credential for those who wish to attend business school, law school, medical school or [go into] a number of other fields[.] … If the *Gathering Storm* report is incorrect, we will end up having devoted additional dollars to improving our children's education and to the discovery of new knowledge. On the other hand, if Drs. Lowell and Salzman are wrong, America may well face a serious growth in unemployment and a commensurate decline in its standard of living."

Those who argue for strengthening U.S. science education say that NAEP is not the right yardstick for measuring what today's students need to know. "In a global economy with a global labor pool, it is insufficient to compare American students' past performance to American students' current performance," says Bill Bates of the Council on Competitiveness, one of several groups that lobbied heavily for the COMPETES Act. Salzman and Lowell say that they are not arguing for the status quo but rather that any new policies should address the real problems in STEM education. For elementary and secondary schools, they call for more resources for the lowest performing students, many of whom are minorities. And within higher education, they say that scholarships should be based on market demand for workers trained in individual disciplines rather than across-the-board support. Salzman also recommends that universities put greater emphasis on teaching communications and teamwork skills. "The iPod's success has had more to do with its creative design rather than its technical guts," he says.

Augustine says Salzman and Lowell have raised some important issues but that he is worried their criticism could undermine efforts to boost the research and training budgets of federal research agencies slated for growth in the COMPETES Act. However, David Goldston, the top staffer on the House Science Committee before he retired from the government last year, doesn't think their paper will weaken the case for greater investments in science and engineering. "It's worthwhile to debate what the nature of the investments should be, what part of the social scale they should be targeted toward, and what competitiveness really comes from," he says. If the new study sparks those discussions, Goldston adds, "that's all to the good."

–YUDHIJIT BHATTACHARJEE

* www.urban.org/UploadedPDF/411562_Salzman_Science.pdf

Against the grain. Harold Salzman (*center*) told Congress last week that the United States produces enough technical workers for the economy.

CREDIT: COMMITTEE ON SCIENCE AND TECHNOLOGY, U.S. HOUSE OF REPRESENTATIVES

Bond slayer. Steven Lonegan (in necktie) pumped up voters' fiscal worries to help defeat the stem cell initiative.

CREDITS (TOP TO BOTTOM): AFP; P. BAGLA

U.S. STATE ELECTIONS

New Jersey Rejects Bonds For Stem Cell Institute

A proposal to ratchet up stem cell research in New Jersey was defeated last week by an array of hard-working opponents and a sense of overconfidence by supporters. Despite recent polls showing that the $450 million bond issue would be approved, the state's voters last week rejected it by a margin of 53% to 47%. "[We] were a little bit too confident" and didn't shift into high gear until too late, says Martin Grumet, director of the W. M. Keck Center for Collaborative Neuroscience at Rutgers University in New Brunswick.

The bond measure, which was on a ballot featuring contests for various local and state offices, would have supplied an additional $45 million a year for stem cell research over the next 10 years to researchers at both public and private entities in New Jersey. It was backed by a succession of New Jersey governors; the incumbent, Jon Corzine, even donated $150,000 of his own money to the effort to get it passed. Last year, Corzine signed into law an allocation of $270 million—New Jersey's share of a national tobacco settlement—for new stem cell research facilities. Of this, $150 million is for the Stem Cell Institute of New Jersey in New Brunswick, for which ground was broken last month.

As recently as October, polls were predicting a comfortable win. But a combination of religious and fiscal conservatives carried the day. Catholic churches showed a video disparaging embryonic stem cell research, and Bishop John M. Smith of Trenton sent out a letter on 7 October urging Catholics—who make up 43% of New Jerseyites—to pray against the referendum. Antiabortion groups nicknamed the measure "Loan to Clone."

The measure also drew the ire of a group called Americans for Prosperity that wants to curb government spending. Steven Lonegan, former mayor of Bogota, New Jersey, who heads the group's state chapter, says the issue caught fire in the past few months. "We engaged more people on the ground than any campaign in the state in a long time," he says. Citing the state's high tax rates and $30 billion debt, the group offered voters a simple message about three fiscal proposals before them: "Vote No on All Ballot Measures."

Rutgers neuroscientist Wise Young, co-founder of the stem cell institute, says that the referendum was fatally hurt by the record-low turnout of 26.6% of eligible voters. Participation was lowest in counties where support for stem cell research was highest, he added.

Corzine says he will continue to press for more money for stem cell research, both from public funds and the private sector. New Jersey, the first state to direct funds to stem cell research, has spent $15.2 million since 2005, according to the New Jersey Commission on Science and Technology, with $10.7 million budgeted for the current fiscal year. Young says he hopes pharmaceutical companies, which have a heavy presence in the state, will pitch in.

"We will have to take a different strategy, … but we're pushing ahead," says Grumet. Supporters regard the defeat as an expression of taxpayer frustration rather than rejection of the research itself. Young says advocacy groups are already rallying around the idea of another referendum next November, to coincide with the presidential and congressional races. And they vow to be prepared this time. "It's going to be a huge fight all the way down the line," says Young.

–CONSTANCE HOLDEN

SCIENCESCOPE

New Limits on Defense Grants

U.S. lawmakers last week put the squeeze on universities that receive basic-research grants from the military by tightening the amount of money that universities can be reimbursed for the cost of facilitating that research. The new language limits overhead costs to 35% of the total amount of the grant. That's the equivalent of a 54% indirect cost rate, and many schools won't notice the difference because their rates do not exceed that amount.

But Barry Toiv of the Association of American Universities says at least 40 universities could be affected by the decision, and lobbyists fear that Congress might eventually apply the same formula to the government's entire research portfolio. "A cap on reimbursement is a first step down a potentially slippery slope," says Toiv. "Preventing universities from reimbursing all the real and necessary costs of conducting research will discourage them from applying for defense grants."

–YUDHIJIT BHATTACHARJEE

Moonstruck

India and Russia have signed an agreement to jointly explore the moon. As a first joint venture, India envisages using one of its rockets to launch a crewless mission in 2011 called Chandrayaan-II (a second moon mission for India). Together, Russia and India will develop a robotic rover that will be deployed from a lunar orbiter to probe the moon's surface for geological data and look for helium-3, a potential fuel for fusion reactors. The collaboration is "an area of great promise" for the two countries, said Indian Prime Minister Manmohan Singh at a meeting in Moscow this week with Russian President Vladimir Putin.

The predecessor to this mission is India's Chandrayaan-I, a resource-mapping project that India plans to launch on its PSLV rocket (above) in April 2008. It will carry two American research payloads and involves no Russian participation. However, India and Russia have collaborated on space projects for decades: India's only astronaut, Rakesh Sharma, lifted off in a Russian Soyuz rocket in 1984, and Russia supplied the cryogenic engines that have carried India's heavy communications satellites into space.

–PALLAVA BAGLA

Chilling conclusion. Rapid arctic melting has stimulated interest in geoengineering.

CLIMATE CHANGE

Scientists Say Continued Warming Warrants Closer Look at Drastic Fixes

CAMBRIDGE, MASSACHUSETTS—Should scientists study novel ways to alter Earth's climate to counteract global warming? Last week, a group of prominent researchers who gathered here gave a qualified "yes"—after agreeing that the road to understanding the science is fraught with booby traps and that deliberately tinkering with the climate could make the problem worse. Some even admitted to being surprised by their affirmative answer.

"My objective going [into the meeting] was to stop people from doing something stupid," says climate modeler David Battisti of the University of Washington, Seattle. But rising temperatures and carbon emissions, combined with little meaningful action by politicians, convinced him and his colleagues that it was time for mainstream climate science to look more closely at geoengineering. Even so, Battisti suspects that the participants share the hope of many of those who took part in the Manhattan Project to build the atom bomb: that society would never have to use the knowledge they provided. "It would be incomprehensible that we deploy this," Battisti says, emphasizing the greater need to cut carbon emissions.

Organized by the University of Calgary and Harvard University, the event allowed 50 elite climate, energy, and economics researchers to explore and debate geoengineering. For decades, the subject has been mostly confined to the pages of science fiction and unfunded by research agencies. But a 2006 paper in *Climatic Change* by Nobelist Paul Crutzen (*Science*, 20 October 2006, p. 401) served as an "enabler" to drive discussion among scientists of the once-taboo topic, says Harvard environmental chemist Scot Martin. Harvard geochemist Daniel Schrag and physicist David Keith of the University of Calgary in Canada then decided to organize the Cambridge event.

One reason most scientists have been leery of probing the topic was the fear that if such technical fixes were taken seriously, public support for cutting carbon emissions would be even more difficult to achieve. "The very best would be if emissions of the greenhouse gasses could be reduced so much that the [geoengineering] experiment would not have to take place," Crutzen wrote last year. "Currently, this looks like a pious wish."

Some scientists, however, have been thinking about geoengineering for quite some time. The field's roots lie in dueling Soviet and U.S. weather-modification programs of the 1960s. Since then, advocates have dreamed up schemes to fight warming by blocking sunlight with giant space shades or by creating sea clouds to increase the albedo of the ocean. In 1997, physicist and Star Wars stalwart Lowell Wood and colleagues affiliated with Livermore Berkeley National Laboratory suggested using aerosols to mimic the cooling effect of volcanoes, and a handful of modeling papers since have simulated that effect.

One of Wood's central points is that the aerosol method is cheap. In 1992, recalls Harvard physicist Robert Frosch, a National Academies' panel on climate resisted his suggestion to include the cost of geoengineering options in a figure on possible solutions to global warming. One relatively simple option: Inject sulfur dioxide into the stratosphere to reduce the amount of solar energy reaching Earth's surface. "Nobody wanted to put the geoengineering line on the figure because it looked too [economically] easy," Frosch told participants.

That cost was a major factor behind the discussions here, with a number of preliminary technical studies hinting that the SO_2 option could be deployed for a few billion dollars a year. That amount could make geoengineering attractive to politicians looking for radical fixes in a warming world. "The decision on whether to do this will not be made by this group," Schrag told his colleagues sitting in the wood-paneled premises of the American Academy of Arts and Sciences. But what scientists can do, he said, is offset the input of groups driven by profit or ideology with solid research on the possible side effects of various geoengineering techniques.

A sea change? Some scientists have proposed creating white clouds over the oceans to help cool the globe.

And to get started, the group certainly suggested plenty of side effects. Atmospheric dynamicists attacked the few modeling studies that have simulated geoengineering efforts for downplaying details such as ocean currents or complex feedbacks. (Modelers defended their studies, which use simplified models, as preliminary.) Ecologists pointed out that artificial cooling could lead to serious drying in the tropics and that any fix that ▶

CREDITS (TOP TO BOTTOM): NASA; (ILLUSTRATION) JOHN MACNEILL

lowers Earth's temperature wouldn't address the problem of the steadily acidifying ocean.

Modeler Raymond Pierrehumbert of the University of Chicago in Illinois warned that geoengineering could become a global addiction. "I don't actually work on geoengineering," he told the group. "But now that the genie's out of the bottle, I feel I have to." In one unpublished experiment, Pierrehumbert simulated a future scenario, presumably in the next century, in which the amount of atmospheric CO_2 had quadrupled but Earth was kept cool by a yearly dose of geoengineering. His model showed that a halt in the geoengineering effort—"by, say, a war or revolution"—would result in an 7°C temperature jump in the tropics in 30 years. That rise, he says, would trigger unimaginable ecological effects.

Sallie Chisholm, an MIT biological oceanographer, urged caution. She told *Science* that her colleagues are downplaying the difficulty of determining how "inherently unpredictable" biospheric feedbacks will react to "turning the temperature knob. … We cannot predict the biosphere's response to an intentional reduction in global temperature through geoengineering."

Other scientists were more willing to entertain the idea of studying climate manipulation but warned about a likely public backlash. Political scientist Thomas Homer-Dixon of the University of Toronto in Canada talked about street protests. "Some people may consider geoengineering to be an act of ultimate hubris," he says. "It's going to provoke fear, anger, guilt, and despair."

Others, however, viewed public alarm about geoengineering as a potentially positive effect. "If they see us talking about this as a last-ditch effort, it might increase their alarm" and drive them to cut emissions, explained Harvard climate dynamicist Peter Huybers during one of the sessions. By the end of the 2-day event, participants were stunned that they had come so far. "In this room, we've reached a remarkable consensus that there should be research on this," announced climate modeler Chris Bretherton of the University of Washington, Seattle. Nobody dissented.

Mixed in with his new sense of "responsibility," Battisti says, is dismay that the climate problem has grown so serious as to drive scientists to contemplate steps that, in theory, might lead to more serious problems than continued warming. After speaking on the phone with his wife from his hotel room, Battisti confessed, "I told her this meeting is terrifying me."

(For a discussion of the topic with some of the meeting participants, go to www.sciencemag.org/hottopics/geoengineering.)

–ELI KINTISCH

BEHAVIOR

Robot Cockroach Tests Insect Decision-Making Behavior

Science-fiction writers have long envisioned societies in which the boundaries between humans and lifelike droids blur and man and machine freely intermingle. José Halloy has taken the first steps toward creating that world, at least for insects. His tiny, autonomous robots lack legs, wings, and antennae, but they nonetheless pass muster with cockroaches. Indeed, these wheeled machines are so well accepted by the household pests that the robots become part of the insects' collective decision-making process, Halloy, a theoretical biologist at the Free University of Brussels, Belgium, and his colleagues report on page 1155. The robots persuaded many of their insect "peers" to hide in an unconventional place.

Halloy's innovative approach puts theories of collective behavior among insects into practice. "We can manipulate these behaviors very easily in a model, but doing so in experiments is often challenging," explains ethologist Jerome Buhl of the University of Sydney, Australia. Others have used remote-controlled robots to study animal behavior but not autonomous ones that interact with animals on their own. "In many ways, [the work] is a big step in the study of collective behavior in animals," says animal behaviorist Stephen Pratt of Arizona State University in Tempe.

Halloy and his Brussels colleague Grégory Sempo picked cockroaches for these robot experiments in part because they had earlier found that cockroaches typically self-organize; within a few hours, for example, they settle together in one place, preferring darker spots when available. For those experiments, and the later ones with the robots, Halloy, Sempo, and their colleagues built a 1-meter-diameter arena with two "shelters," the roofs of which were made of plastic discs covered by red filters. By adding layers of filters, Halloy and Sempo can make one shelter darker than the other.

Based on observations of insects in this arena, Halloy and his colleagues developed a mathematical model that predicts which shelter a cockroach should pick depending on the level of darkness of the shelter and the number and activity of its fellow roaches. Halloy's group then used this model to program robots designed by him and Francesco Mondada and other engineers at the École Polytechnique Fédérale de Lausanne, Switzerland.

The roaches usually ran away from the robots but not if the machines smelled like the insects. For the experiments, Halloy and Sempo covered the robots with a filter paper containing the pheromone equivalent of one cockroach.

Halloy initially programmed the robots to have the same darkness preference as the cockroaches, and they joined the cockroaches at whatever shelter the majority chose to rest in. Next, Halloy programmed the robots to prefer the lighter shelter. About 60% of the time, the robots tipped the group's preference in favor of the light shelter. "This is a true example of automated leadership," says David Sumpter of Uppsala University in Sweden. "Instead of the robots rounding up the cockroaches like sheepdogs, they lead through social attraction."

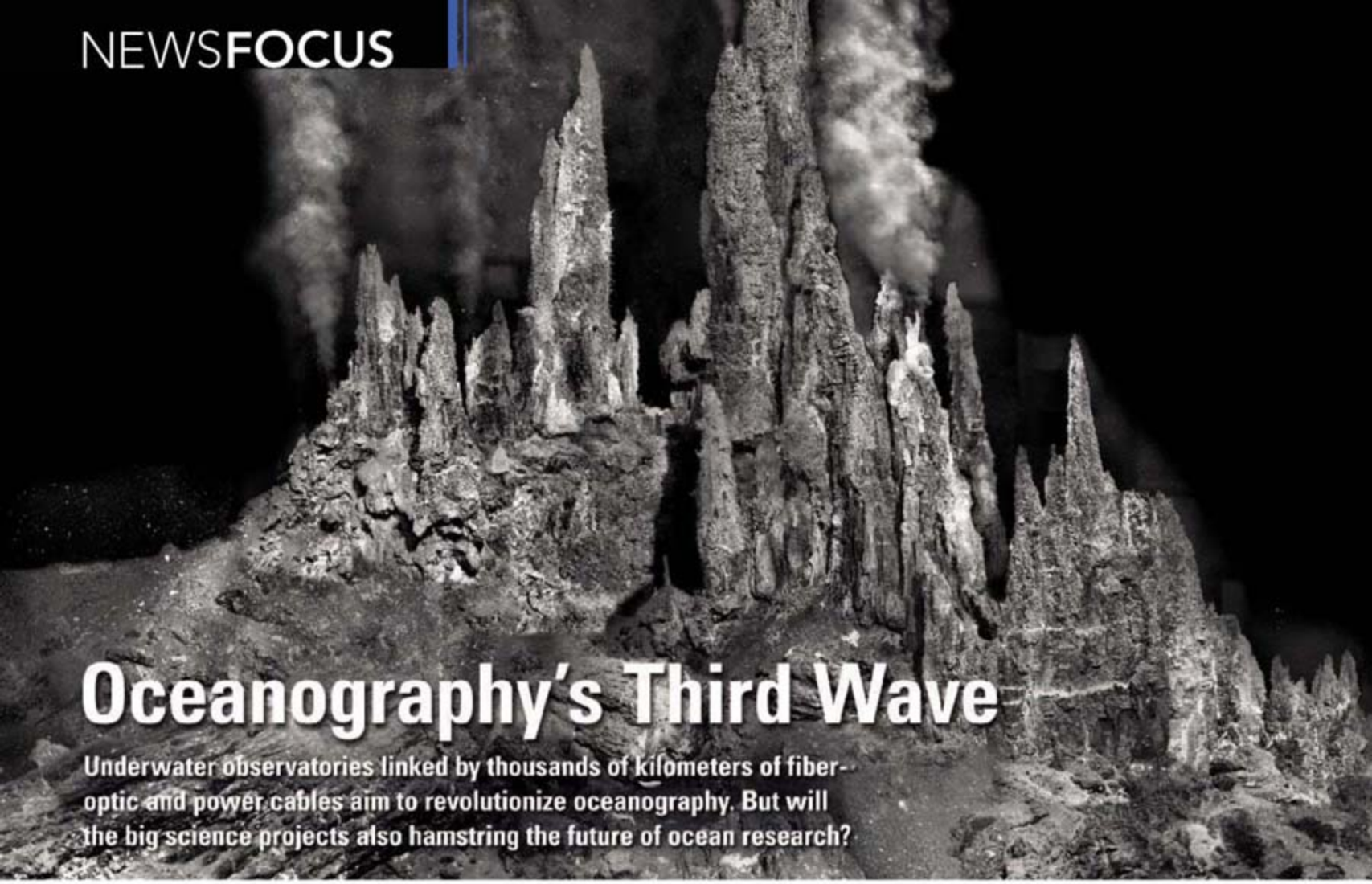
Can't we be friends? Cockroaches seem to accept this robot as one of their own once it's coated with pheromone.

CREDIT: ULB-EPFL

But Coby Schal, an urban entomologist at North Carolina State University in Raleigh, has reservations about the effectiveness of the pheromone guise in convincing the roaches that the robot is just like them. He wonders if the physical presence of the robots made the lighter shelter more attractive simply by increasing the structural complexity of this hiding place. "In my view, the jury is still out" on whether the robots became part of the decision-making, says Schal.

Nonetheless, roboticist Daniela Rus of the Massachusetts Institute of Technology in Cambridge calls the idea that robots can influence biological group behavior "very powerful." She speculates that the work could have many applications, such as robots that aid pest control by luring insects into traps or that help herd livestock.

–ELIZABETH PENNISI



Oceanography's Third Wave

Underwater observatories linked by thousands of kilometers of fiber-optic and power cables aim to revolutionize oceanography. But will the big science projects also hamstring the future of ocean research?

SEATTLE, WASHINGTON—When John Delaney first started using ships and submersibles to explore the underwater volcanoes off the coast of Washington state back in the early 1980s, the experience, he says, was exhilarating. Delaney was part of the team that in 1984 discovered the Endeavour vent, a 70-kilometer-long volcanic ridge where magma from Earth's mantle wells up between a pair of tectonic plates. As exciting as those research trips were, they were equally exasperating, says Delaney, an oceanographer at the University of Washington (UW), Seattle, who looks every inch the bearded sea captain and is fond of reciting T. S. Eliot, Ralph Waldo Emerson, and Robert Frost. "They offered snapshots," he says. "We would get a ship and sub for a couple of weeks, come home, publish our results, and then write another set of grants. We would go and see something interesting. But it was 2 or 3 years before we could come back and see what was going on. That became very frustrating."

Delaney's frustration convinced him that there had to be a better way—a means to set up a sustained research presence in the ocean. Ocean buoys outfitted with sensors of course have continuously monitored conditions such as sea-surface temperatures for decades. But that was harder to do 1000 meters or more below the ocean's surface, and deep-water devices suffered from weak power supplies and either could transmit only small amounts of data back to shore or had to be retrieved after months of data collection.

In 1987, Delaney hatched the idea of using underwater telecommunications cables to wire the sea floor. Such cables already crisscrossed the ocean carrying phone calls and computer data between continents. If researchers could tap into those cables, perhaps they could use them as both a power source and a conduit to link to a new generation of sensors, robots, and autonomous vehicles. The idea lay dormant for a few years but slowly started to gain steam. "It was like a snowball going downhill," Delaney says. "People said, 'If you are going to do that, then I can hook into it to look at fish stocks, tsunamis, pollution, and so on,' " he says. In 1998, Delaney successfully pitched the idea for a feasibility study to the National Oceanographic Partnership Program, which fosters collaborations among U.S. federal agencies, universities, and companies to promote ocean issues. At about the same time, researchers at other major oceanographic institutions such as the Scripps Institution of Oceanography (SIO) in San Diego, California, and the Woods Hole Oceanographic Institution (WHOI) in Massachusetts also began pushing the same notion.

Now, after dozens of meetings, reports, and reviews, ocean scientists are setting up a handful of deep-water cabled observatories and are gearing up for a new wave of ocean research. Beginning in 2010, researchers from UW, SIO, WHOI, and Oregon State University (OSU) in Corvallis plan to string cables to sites scattered around an entire continental plate—the Juan de Fuca Plate off the coasts of Oregon and Washington state (see figure, p. 1057). Cable was recently laid for a Canadian arm of the project as well as for a deep-water cabled-observatory test bed off the coast of Monterey, California. Early next year, the European Union will link instruments to three separate cables currently being used to wire up underwater neutrino observatories, and its members are considering further dedicated cable systems down the road. And Japan and Taiwan have recently installed cabled systems, largely for seismic research.

"It's really a dawning of a new age of how humans can explore the oceans," Delaney says. Ship-based oceanography began in the 1870s, when the British ship *Challenger* conducted the first-ever prolonged oceanographic cruise, he notes. Satellites gave oceanographers a

CREDIT: J. DELANEY AND D. KELLEY, SCHOOL OF OCEANOGRAPHY, UNIVERSITY OF WASHINGTON

High definition. Cabled observatories will scrutinize ocean hot spots such as these black smokers.

global reach about 100 years later. "What we're looking at now is a third phase," Delaney says. It's one in which continuous power and data channels offer researchers the ability to develop a new array of instruments that can carry out tasks as wide-ranging as continuously monitoring the steady stream of microtremors at a mid-ocean ridge or sequencing the DNA of underwater organisms on the spot and shipping the data back to researchers continuously. Until now, the ocean has been close to a black box, says Marcia McNutt, president and CEO of the Monterey Bay Aquarium Research Institute (MBARI) in Moss Landing, California. "I will be very surprised if we do not make startling new discoveries, because we will be there 24/7 and can study the ocean on our terms," McNutt says.

But although proponents of the system are quick to liken the utility of the new cabled observatories to the revolution satellites provided to oceanography, detractors say a more apt comparison is the international space station: a money sink that provides scientific value to relatively few researchers. The concern, they say, is that the cabled observatories will be so expensive to maintain and operate that they will inevitably siphon money from other areas of ocean sciences. "Ocean scientists are always of one mind when it comes to ships," says Peter Niiler, a physical oceanographer who retired this summer from SIO. "We are usually of one mind when it comes to satellites. We are not of one mind on this."

Growing appetite

While Delaney has spent decades waiting to complete his vision of a cabled underwater observatory, others have beaten him out of the gate. In 1992, researchers led by physical oceanographer Scott Glenn of Rutgers University in New Brunswick, New Jersey, launched a near-coast cabled observatory called LEO-15, which was built off the coast just north of Atlantic City, New Jersey, in water 15 meters deep and thus could be serviced and maintained by scuba divers. "It really whetted the appetite of the science community to do more," McNutt says.

Researchers in Hawaii took the next step in 1998, when they were given use of an abandoned undersea telephone cable that runs from the Hawaiian island of Oahu to California. They made it the backbone of the first deep-water cabled monitoring system, known as the Hawaii-2 Observatory. Below 5000 meters of water, researchers inserted a junction box with eight ports for tapping into the cable's power supply and hooked up a variety of seismometers, pressure sensors, and a hydrophone. The network operated for only 5 years but helped persuade oceanographers to push for purpose-built cabled observatories.

Wired. Oceanographers plan to use telecommunications cable to supply power and data connections to instruments in the northeast Pacific.

Such observatories, say Delaney and other proponents, have two big advantages. "What these provide is unlimited power and the ability to get data back to shore in real time. That's fabulous," says McNutt. Ocean sciences instruments—including buoys at the surface and seismometers and other sensors on the sea floor—have traditionally worked with very limited power, supplied either by batteries or by small wave-powered generators and the like. Therefore, they typically haven't been able to record or transmit large amounts of data. That has tended to limit them to taking periodic measurements that were either sent back to shore by a low-bandwidth satellite connection or archived onboard for retrieval months later. "If you think how controlled we have been by the whims of the ocean, this takes away those limitations and will allow us to say for the first time what really happens during hurricanes, earthquakes, and other events," McNutt says.

McNutt, Delaney, and other proponents say that cables now being laid off the coasts of California and British Columbia will give researchers the first glimpse of oceanography's future. Off California's coast, the 52-kilometer Monterey Accelerated Research System cable—about the width of a garden hose—will carry fiber-optic data and 10,000 volts of electricity to science "nodes," essentially underwater transformers that reduce the voltage and sit alongside eight ports. Instruments will connect directly to the ports or be linked to them by underwater extension cords.

The instruments vary widely. One, called the Eye in the Sea, will use a video camera that amplifies stray photons to image deep-water bioluminescent organisms. Another, a robotic microbiology lab, will analyze DNA and RNA samples to determine which organisms are present and possibly even discover new life forms. Yet another—known as the benthic rover—is a robot the size of a riding lawn mower that will creep across the ocean floor taking measurements in an effort to sort out the long-standing mystery of just how much organic carbon drifts down from above and reaches the deep ocean floor.

Because these and other such experiments are power-hungry and typically operate continuously, they represent science that can't be done with traditional instrumentation. Another advantage, says marine biologist Ken Smith of MBARI, who heads the team of scientists working on the benthic rover, is that if researchers spot something interesting in the data one day, the next day they can reprogram their instruments on the fly to monitor it. "I'd love to have cabled observatories all over the sea floor," Smith says.

Vigorous discussion

Despite their obvious upside, cabled observatories have long proven a tough sell. The issue, Niiler says, stems from the wide

CREDIT: THE IMPLEMENTING ORGANIZATION FOR THE REGIONAL CABLED COMPONENT OF THE OCEAN OBSERVATORY INITIATIVE, UNIVERSITY OF WASHINGTON

Bottom to top. Power will enable new deep-water robots as well as instruments working all the way up to the surface.

diversity of disciplines in the ocean sciences, including geologists and geophysicists interested in understanding plate tectonics, fisheries biologists gauging fish stocks, and physical oceanographers interested in tracking how carbon dioxide moves between the atmosphere and the oceans. "Oceanography is not one science," says Niiler. "It's a catchall for scientists that need a common facility that is unique, which is ships."

In the United States, however, those communities essentially compete for one pot of money, as the lion's share of funding for basic ocean-sciences research comes from the National Science Foundation (NSF). This year, Congress gave NSF $5 million to begin construction of the $331 million Ocean Observatories Initiative (OOI); NSF hopes for a big funding spike in 2009–11 to finish the job. The largest portion of funds—estimated to be about $170 million—will go to build the deep-water cabled observatory off the Oregon and Washington coasts. When the idea for this expensive observatory first began to gain traction several years ago, the broader oceanography community balked until additional components—a few buoys designed to operate at high latitudes, a network of instruments off the Oregon coast, and a cyberinfrastructure component to handle the expected surge of data—were added. "In order to get community buy-in to the OOI idea, there were some compromises made," McNutt says.

However, although Congress agreed to pay for the new infrastructure, it didn't provide any extra money to run or maintain OOI's cables and instruments. That money—expected to total about $50 million a year—will have to come out of NSF's general budget of about $300 million a year for ocean sciences. Niiler and other critics argue that because the cabled observatories are fixed in place on the ocean floor, they will primarily benefit underwater geologists and geophysicists. Yet, because the operations and maintenance funding will come from the ocean-sciences community as a whole, the high price tag could force cuts in other areas. "I worry that small, individual principal investigator–driven science will be harmed in its breadth and depth by paying too much for these observatories," says a U.S.–based oceanographer who asked not to be named out of concern that it could hurt his chances of acquiring future NSF funding.

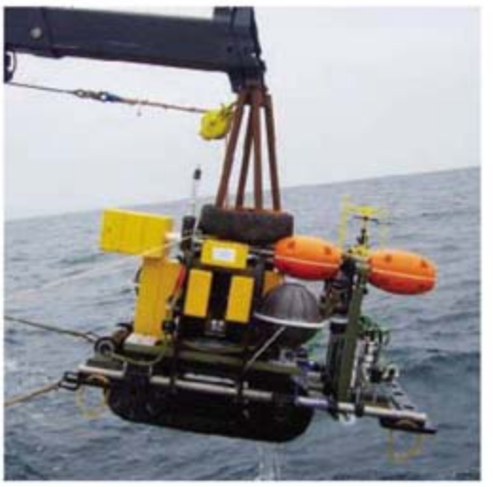
"We agree that operations and maintenance funding is the ongoing struggle of science right now," says Adam Schultz, a geophysicist at OSU, who is currently serving as a program director for ocean sciences at NSF. However, he and others point out that geologists and geophysicists aren't the only ocean scientists getting new equipment these days. Other researchers have received, from NSF and other sources, a $120 million ship for arctic research, $100 million for a new drilling ship, submersibles, and a global ocean float network called Argo. "Now we are adding new capability that will not appeal to everyone. As an organization, we have to find a balance," Schultz says. Julie Morris, director of NSF's ocean sciences division, adds that if Congress and President George W. Bush continue their push to double spending on physical sciences research and development, that could obviate much of the funding concerns.

For his part, Delaney is quick to deny that deep-water cabled observatories are of little interest to anyone but geologists. "I flatly disagree," he says. "There is a tremendous amount of oceanographic science to be done in the waters overlying the Juan de Fuca Plate." Niiler acknowledges that a cabled observatory offers advantages for studying ocean vents and the chemistry and organisms in the waters around them. But if you're interested in how the exchange of gases between the ocean and atmosphere affects climate change, he says, the cabled observatory has far less relevance. "They just don't go together," Niiler says. "To pretend they do is just not right."

Critics also fear that once major new observatories are built, future research grants will tilt in favor of scientists who propose to work on them. "It will be hard for [funding agencies] to resist the temptation to feed this big facility," says the anonymous U.S.–based oceanographer. "It's exactly like the [international] space station," adds Russ Davis, another oceanographer recently retired from Scripps. "We will have to have guys use this thing once we put this together."

Delaney and others respond that all the scientific projects associated with the cabled observatories will be peer-reviewed, although Delaney readily acknowledges that the oceanography community is still coming to grips with the facilities. "It's a vigorous discussion, as it should be," he says. Christoph Waldmann, a member of the European Sea Floor Observatory Network steering committee looking into a new European cabled-observatory system, says the dialogue under way in Europe and elsewhere is much the same. But he and others repeat that proponents of cabled observatories are not out to do away with anyone else's research but rather seek to open dramatic new possibilities to ocean research. "The science community is saying this is a capability we need to have," says Robert Detrick Jr., a marine geologist at WHOI who chaired a recent National Research Council report that favored building cabled observatories. "They provide a new capability for the community and approach ocean sciences in a different way."

–ROBERT F. SERVICE



Creeper. This robotic rover will track how much organic carbon reaches the sea floor.

CREDITS (TOP TO BOTTOM): THE IMPLEMENTING ORGANIZATION FOR THE REGIONAL CABLED COMPONENT OF THE OCEAN OBSERVATORY INITIATIVE, UNIVERSITY OF WASHINGTON; KEN SMITH/MBARI

PUBLIC HEALTH

In the HIV Era, an Old TB Vaccine Causes New Problems

The only TB vaccine available can be deadly for HIV-infected children. That puts public health officials in a difficult dilemma

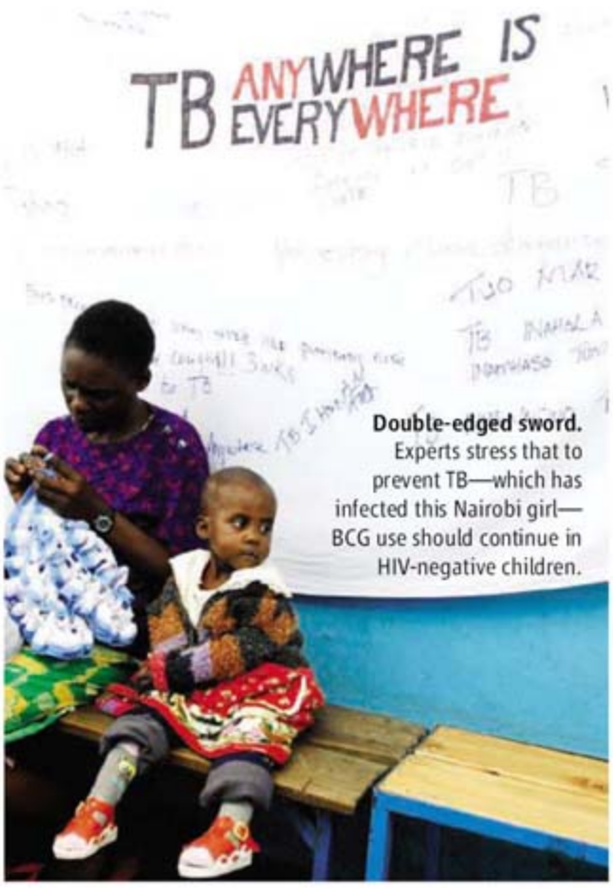
It's well-known that HIV and tuberculosis (TB) form dual, deadly epidemics that fuel each other. More than 13 million people, most of them in sub-Saharan Africa, are infected with the pathogens that cause both diseases. Recently, researchers have found that HIV-infected children—who need protection from TB more than anyone else—are also much more susceptible to side effects of a widely used anti-TB vaccine. The live vaccine, developed more than 80 years ago and known as Bacille Calmette-Guérin (BCG), can lead to a generalized infection that may be fatal in as many as 75% of cases.

Based on these data, experts agree that no baby with HIV should be vaccinated against TB. But that's easier said than done; identifying infected infants isn't feasible in many countries with high HIV rates. What's more, researchers worry that discussing these side effects could give the vaccine a bad name and lead to a drop in overall vaccination rates. "It's really a terrible dilemma," says pediatrician Elizabeth Talbot of the Dartmouth-Hitchcock Medical Center in Lebanon, New Hampshire.

As vaccines go, BCG was always a mixed blessing. Researchers at the Pasteur Institute in Paris developed it by weakening a strain of *Mycobacterium bovis*, a cousin of *M. tuberculosis*, the agent that causes human TB. (The process was inspired by vaccinia, the smallpox vaccine most likely derived from cowpox.) BCG doesn't appear to do much for adults, but it protects children from the most serious forms of TB during the first 15 years of life, although efficacy varies somewhat in different parts of the world.

BCG is the only TB vaccine that exists, and almost every country in the world uses it. Recently, many Western countries have started limiting their use to high-risk groups, such as immigrants from endemic countries, because dropping TB rates have made the vaccine less cost-effective. But in regions with high TB rates, it's still an important line of defense. In Africa, most children are vaccinated right after birth. Some studies have even shown that BCG reduces mortality from causes other than TB as well, perhaps because it boosts the immune system.

Researchers have long known that BCG could cause adverse events in immunocompromised people, ranging from local reactions to disseminated BCG disease, a life-threatening infection. "But until now, we didn't have any solid data" on the magnitude of the problem, says T. Mark Doherty of the Statens Serum Institute in Copenhagen, Denmark. In areas with high HIV rates, babies are prone to so many other diseases—including TB, malaria, and gastrointestinal infections—that a vaccine-specific reaction is hard to notice. And diagnosing BCG disease requires culturing the bacteria from infected children—a time-consuming process—and then using a polymerase chain reaction test to determine that they are *M. bovis*, not *M. tuberculosis*.



Double-edged sword. Experts stress that to prevent TB—which has infected this Nairobi girl—BCG use should continue in HIV-negative children.

CREDIT: DAMIEN GUERCHOIS/REUTERS/LANDOV

So when Anneke Hesseling of the Desmond Tutu TB Centre of Stellenbosch University near Cape Town, South Africa, started looking carefully in a hospital in the Western Cape province, where both HIV and TB are rampant, the outcome came as a shock to many. In a paper published in *Vaccine* in January, Hesseling concluded that disseminated BCG disease may occur in one in every 240 HIV-infected vaccinees at that hospital; that's more than 500 times the risk for healthy children.

A new, unpublished study based on better surveillance in more hospitals suggests that the risk for HIV-infected children may be two times higher still, she adds. The concerns about BCG are corroborated by an as-yet-unpublished study in Argentina, presented at a 2005 meeting by Aurelia Fallo of Children's Hospital Ricardo Gutiérrez in Buenos Aires.

The solution sounds easy: Children born to HIV-infected mothers should be tested, and if HIV-positive, should not get BCG. But in many African countries, mothers aren't tested for HIV to begin with. What's more, mother-to-child transmission of HIV occurs mostly around birth, so tests can't be reliably done until at least 6 weeks later. That would mean postponing the decision to vaccinate HIV-exposed children during that period.

The Western Cape is considering a program to do just that, says Hesseling, but it's difficult enough in South Africa; in many other African countries, it's likely not feasible. Any change in standard vaccination schedules is a major undertaking, she says, and postponing vaccination carries the risk that some of the children will never come back. Even in countries with high HIV rates, a large majority of children are HIV-negative, she points out; if they missed the vaccine because of a policy change, "it would be a disaster."

Many are looking to the World Health Organization (WHO) for guidance. In May, after reports from two expert panels, WHO began advising against vaccinating HIV-positive babies. But the new recommendations are "not all that helpful," says Hesseling. In an e-mail to *Science*, a WHO communications officer said that "there is concern that recommendations might become counter-productive if BCG use ceases or is discontinued in HIV-endemic populations." WHO continues to recommend using BCG if nothing is known about a child's HIV status, she wrote.

The agency is in a difficult position, Doherty says, as it will be blamed if overall vaccination rates drop. "In situations like this, there's always a tendency to err on the side of the status quo," he says. **–MARTIN ENSERINK**

ROBOTICS

Robotic Cars Tackle Crosstown Traffic—and Not One Another

In DARPA's Urban Challenge, cars that drive themselves face off in a strange, soulless rush hour. Are human drivers about to go the way of the buggy whip?

VICTORVILLE, CALIFORNIA—The Land Rover bristles with sensors like a mechanical porcupine. John Leonard, an engineer at the Massachusetts Institute of Technology (MIT) in Cambridge, ticks off the robot's features. On the roof spins a conical laser range finder called a lidar that sees in three dimensions. A dozen lidars that see in one direction, 15 radars, and six digital cameras look out every which way. Computers fill the back of the truck, and a generator supplies the 3.5 kilowatts of power they need. It's impressive. But all this so the truck can turn left across traffic by itself?

The robot is one of nearly three dozen vying in the Urban Challenge, a competition sponsored by the U.S. Defense Advanced Research Projects Agency (DARPA). It's the third and most demanding in a series that aims to spur the development of autonomous vehicles, which the U.S. military hopes to press into service by 2015. In 2004 and 2005, robots raced one at a time across open terrain. This time, they must navigate the streets of an abandoned air base here in the Mojave Desert without colliding with one another or with human-guided vehicles.

The competition showcases some of the world's best talent in robotics. "We were drawn to the Urban Challenge because it requires real-time decision-making in a dynamic environment and in the presence of uncertainty," Leonard says. It also serves the higher purpose of trying to save lives, as worldwide, 1.2 million people die each year in traffic accidents that robotic cars might help avoid.

And yet the Urban Challenge is at least slightly absurd. It looks a bit like a real race. Engineers wear bright shirts emblazoned with the logos of sponsors—GM, Ford, Intel, Google. Teams have hauled in tractor trailers full of equipment and plastered their robots with decals. Besides the $2 million first prize, the appeal of the challenge is obvious. It's hard, and by pitting idea against idea and technology against technology, "it determines what technical DNA moves to the next generation" in the evolution of autonomous vehicles, says roboticist William "Red" Whittaker of Carnegie Mellon University in Pittsburgh, Pennsylvania.

Still, the task the robots will attempt seems so ordinary. They must obey the California traffic laws (although if two collide, they won't have to exchange insurance information as human drivers are required to do). We're all here to watch traffic. But we won't see with our own eyes. Instead, we'll have to watch it on television. It's not even clear what DARPA gets out of this well-crafted media circus. The competition is meant to stimulate the development of cars that drive themselves—and it has—but DARPA does not require winners to reveal to the agency the details of their technologies.

Comeback kid. After a delayed start, Carnegie Mellon's Boss cruises briskly to a victory.

Go ahead, bend the rules

At the decaying fighter base, across the road from the new federal prison here in Victorville, 11 teams have made the final competition. Three years ago, not one robot traversed more than a dozen kilometers of the 230-kilometer off-road course. A year later, four completed a similar course. And this year's robots are far more capable than last year's crop, possessing better sensors, more powerful computers, and, most important, more sophisticated programming. "Driving is a software problem, not a hardware problem," says engineer Michael Montemerlo of Stanford University in Palo Alto, California. "At Stanford, we can't build a better car, but we can make a smarter car."

Computationally, this year's challenge is much more difficult than the first two, researchers say. In the desert races, the robots had only to identify obstacles in a static landscape and plot a safe path around them. This time, the vehicles will have to avoid other cars, including other robots, while at the same time obeying the relatively arbitrary traffic laws. To do that, each robot's computer must calculate the likely trajectories of all the objects around it and plan to miss them. Of course, a robot cannot know exactly where another car will go, so the machines generally employ layers of probabilistic algorithms to decide their next moves.

MIT has decked its robot, Talos, with the most sensors. The radars see distant objects, the lidars see at an intermediate range, and the cameras spot things close by, explains David Barrett, a team member from the Franklin W. Olin College of Engineering in Needham, Massachusetts. Talos depends mainly on its sensors to navigate, Barrett says. That's because the team assumed, incorrectly it turns out, that DARPA would not let robots use signals from the satellite-based Global Positioning System (GPS) all over the course.

Researchers from Stanford, who won the 2005 competition, say that they focus on the algorithms programmed into their robot. Merely encoding the traffic laws can leave the robot stymied, says Stanford computer scientist Sebastian Thrun. For example, when two robots arrive at a four-way stop simultaneously, each may try to yield to the other endlessly. To avoid such deadlock, the team lets its robot skirt the laws. "Our car has a hierarchy it follows," Thrun says. "At the top, it obeys strict rules. And if it gets stuck, it ignores more and more rules." Fair enough. Why expect more from a robot than a human?

Team AnnieWAY, one of two German squads in the final, has taken a minimalist approach to guiding its Volkswagen Passat. The team relies almost entirely on the $75,000 three-dimensional (3D) lidar, which Bruce Hall and colleagues at Velodyne Acoustics Inc. in Morgan Hill, California, developed to compete in the 2005 DARPA challenge. The sensor may be all you need, says Sören Kammel of the Karlsruhe Institute of Technology in Germany. "I think some teams have a lot of sensors because they have a lot of sponsors, and everybody wants their sensor on the car," he says.

Even that one sensor is beyond the means of Donald Harper and his six teammates from

CREDIT: A. CHO/SCIENCE

After you. Stanford's Junior and Virginia Tech's Odin negotiate an intersection.

CREDIT: DARPA

the University of Central Florida in Orlando. They've outfitted Knight Rider, a 1996 Subaru Outback that belonged to Harper's wife and has 99,257 miles (159,705 km) on it, with just enough gizmos to get around the course—they hope. Instead of the spinning 3D lidar, they use two lidars that see in one direction and rock them back and forth. "If just one wire falls off, something essential is not going to work," Harper says. Still, the team made the final having invested only $130,000 in the project.

Robots, start your engines!

Race day usually brings the intoxicating smell of high-octane fuel and the electrifying scream of engines. But not here. At 8:00 a.m., the robots leave the starting area, one by one, like rental cars leaving a lot. There's a glitch. Interference from a jumbo TV screen knocks out the GPS receiver of first qualifier, Boss, Carnegie Mellon's Chevy Tahoe. The team replaces the unit and has to wait 30 minutes to regain the signal. Meanwhile, Odin, a Ford Escape from Virginia Polytechnic Institute and State University in Blacksburg; Junior, Stanford's Volkswagen Passat; and the others head out, hesitating and swerving as if driven by octogenarians. After a half-hour, all 11 robots—plus their chase cars and 37 other cars—are on the road.

There's only one curve from which to glimpse the robots, so DARPA has hired a helicopter and is televising the event on three huge screens in a vast tent. Jamie Hyneman and Grant Imahara of the geeky cable-television reality show *Mythbusters* provide commentary. It's like watching a hybrid of a NASCAR race and the infamous O. J. Simpson low-speed police chase.

Each robot has to complete three "missions" comprising six or seven "submissions," such as parking in exactly the right space in a lot, traversing an off-road passage, or navigating between two places. After each mission, the robots return to the start area to download the specifications for the next, and each machine must travel 60 miles (97 kilometers) in less than 6 hours.

At first, the action comes fast and heavy. An hour into the race, TerraMax, the hulking vehicle entered by military contractor Oshkosh Truck Corp. in Wisconsin, turns toward a pillar and gets stuck staring at it. Forty-five minutes later, Central Florida runs straight toward a house. Caroline, the robot from Team CarOLO, the other German squad, collides with MIT's Talos and loses sensors. By 11:00 a.m., five robots have either failed or been disqualified.

Then things settle down. The remaining robots' "personalities" emerge. Carnegie Mellon's Boss zooms confidently away from stops, a hard charger like team leader Whittaker. Stanford's Junior glides around smoothly, so much so you hardly notice it. MIT's Talos is aggressive in traffic—it also clips Cornell's Chevy Tahoe, Skynet—but skittish off-road, stopping and starting like a cat creeping down a steep slope.

Around 1:30 p.m., three teams have nearly completed their missions, and spectators swarm back to the grandstands. At 1:42, Stanford cruises across the finish line, followed a minute and a half later by Carnegie Mellon. Upstart Virginia Tech cruises home third—even without the 3D lidar. "We knew we were good," says Virginia Tech's Alfred Wicks. "We'd done our homework." The University of Pennsylvania's Toyota Prius, Little Ben, straggles in an hour later. Sometime past 3:30 p.m., MIT slips in just before Cornell.

The outcome seems obvious. Carnegie Mellon spotted Stanford and Virginia Tech a 20-minute head start and made up almost all of it. It seems the victory should be theirs. DARPA officials will make the final call, however. And, some participants grumble, DARPA never fully explains its judgments.

Make it out to ...

But the next morning brings no surprises. Carnegie Mellon walks off with the win. Stanford takes second and $1,000,000, Virginia Tech takes third and $500,000. "There's tremendous satisfaction in what the whole field accomplished," Whittaker says. "That was a day that stunned the world." DARPA Director Anthony Tether also gushes. "Quite frankly, I watched these things and I forgot after a while that there wasn't anybody in there," he says. "It's a historic day—'bot on 'bot for the first time!"

Maybe there's something to the grandiose rhetoric. Now only a Luddite could doubt that soon cars will guide themselves, at least in a pinch to avoid collisions. In fact, the technology already seems ripe for low-risk applications, such as automating farm equipment, and the leading teams are pushing to commercialize their software. "I think it's going to come in bits and pieces," says Charles Reinholtz, leader of the Virginia Tech team and an engineer at Embry-Riddle Aeronautical University in Daytona Beach, Florida.

Ironically, the success of the Urban Challenge could reduce the chances that DARPA will stage another competition. "DARPA never finishes anything," Tether says. "All we do is show that it can be done" in the hope that industry takes over and pushes further development. Clearly, when it comes to making robotic cars, the Urban Challenge has shown that it is possible.

Still, many engineers are eager for another competition. Their robots aren't nearly ready for the open road, they say, and many already know what they would like to see in the next challenge: a contest for autonomous cars that must communicate and work together. Suddenly, that doesn't seem quite so absurd.

–ADRIAN CHO

BEHAVIORAL GENETICS

Evidence Linking *DISC1* Gene to Mental Illness Builds

Animal studies add weight to the view that an important gene for brain development plays a role in diseases such as schizophrenia and depression

Every clan has its misfits, but an extended family in northern Scotland is extraordinary. More than half have suffered from schizophrenia or some other form of mental illness. A group of Scottish researchers reported in 1990 that the affected people all carried the same genetic anomaly—a translocation, or swap, of two stretches of DNA on the long arms of chromosomes 1 and 11. With modesty, the investigators wrote that this "may be a promising area to examine" for genes that predispose people to mental illness.

The area turned out to be very promising indeed. By the year 2000, it had led researchers to a gene called *DISC1*, which may be a key player in the chain of events leading to mental illness. The circumstantial evidence for assigning a major role to *DISC1* (*Disrupted-in-Schizophrenia 1*) is strong. Several studies have linked the gene to schizophrenia, major depression, bipolar disorder, and autism; recent findings on *DISC1*'s biological function appear to support the hypothesis.

Animal studies have shown that the gene is needed for normal brain development both in the embryo and later in life and that blocking its function produces subtle abnormalities in brain structure resembling those seen in patients with schizophrenia. The protein encoded by the gene also turns out to be part of a nerve cell signaling pathway involved in learning, memory, and mood. "I think this gene is really the first big breakthrough in schizophrenia … and other mental diseases," says Christopher Ross of Johns Hopkins University School of Medicine in Baltimore, Maryland.

After decades of following false leads, researchers are cautiously optimistic that they are on the right track with *DISC1*. But the evidence isn't airtight. Except in the Scottish family, researchers haven't consistently linked any particular *DISC1* variant to a mental disease. "There's no smoking gun," cautions psychiatrist Daniel Weinberger of the National Institute of Mental Health in Bethesda, Maryland. But if the connection of *DISC1* to mental disorders holds up, it might lead to better therapies for treating the conditions—especially schizophrenia, a devastating disease that is now poorly controlled at best.

The hunt begins

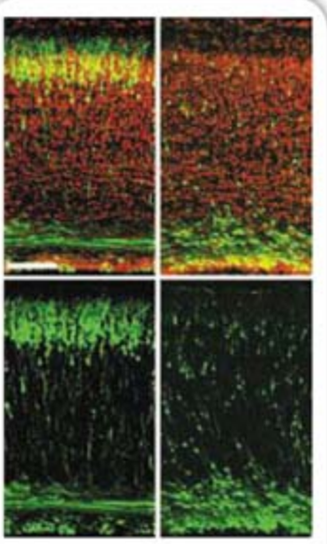
Gene hunters have had a hard time pinning down the genes involved in mental disorders mainly because the diseases are complex, meaning that several genes, as well as environmental factors, contribute to their development. That's why the Scottish family proved to be such a boon. The 1990 study, which was conducted by a team including David St. Clair, Douglas Blackwood, and Walter Muir of the University of Edinburgh, U.K., suggested that the region disrupted by the translocation seen in affected members of the Scottish family held one or more genes involved in the disorders.

The gene search went slowly at first, but in 2000, a team led by David Porteous and Kirsty Millar, also at Edinburgh, identified two previously unknown genes on chromosome 1 that were interrupted by the genetic anomaly. Attention has focused on the first, *DISC1*, which normally produces a large protein the structure of which suggests that it interacts with other proteins.

Shortly after identification of *DISC1*, a follow-up study by the Edinburgh workers buttressed a causative role for the gene in the mental disorders of the Scottish family; that linkage analysis had a high degree of statistical validity—a LOD score of 7 when 3 is considered good. In this family, "we're as close to causality as you could get," Porteous says. Even so, environmental influences may still be important, as a few members carry the translocation but remain unaffected.

The Scottish family is unusual because so many members develop bipolar disorder and depression, as well as schizophrenia, and no one has detected a similar *DISC1* abnormality in other families. In 2003, however, Leena Peltonen of the National Public Health Institute in Helsinki, Finland, and her colleagues reported a linkage between a particular set of three single-nucleotide variants (single-nucleotide polymorphisms) in *DISC1* and schizophrenia in a group of 458 Finnish families. "This is the first genetic evidence that *DISC1* has something to do with the more common garden variety of schizophrenia," Peltonen says.

Other workers have picked up linkages between *DISC1* variants and schizophrenia in a few U.S. and European families. And this year, the Peltonen team linked the gene to bipolar disorder and to autism in their Finnish population.



Held in place. An RNAi that blocks *DISC1* synthesis prevents migration of neurons (green) to the upper layer of the cortex in mice (right micrographs); controls are on the left.

CREDITS (TOP TO BOTTOM): SPENCER JONES/GETTY IMAGES; KAMIYA *ET AL.*, *NATURE CELL BIOLOGY* 7, 12 (2005)

Research on *DISC1*'s normal function has strengthened the case that it is involved in mental disease. For starters, the gene is expressed in many tissues, but particularly in brain areas such as the hippocampus and cerebral cortex that are affected in schizophrenia. That puts the gene's protein product in the right locations to influence the development of the mental disease.

In addition, as predicted from *DISC1*'s structure, researchers have unmasked numerous binding partners for the protein. The current count stands at about 50, Porteous says, including "10 or 12 where the interaction influences function." Several of these partners suggest a role for *DISC1* in brain development and cognition.

For example, about 5 years ago, three independent groups, those of Porteous, Akira Sawa of Johns Hopkins University School of Medicine, and Christopher Austin at Merck Research Laboratory in West Point, Pennsylvania, found that *DISC1* binds to a protein called NUDEL (for NudE-like) that is needed for the neuronal migrations that occur during brain development. Several other partners of *DISC1*, including FEZ1, LIS1, dynein, and tubulin, are also involved in nerve-cell migrations.

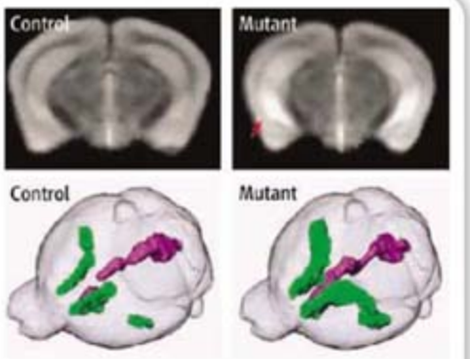
That suggests that brain development might go awry if *DISC1*'s function is altered or missing. Evidence supporting that idea includes the demonstration about 3 years ago by the Sawa team that inhibiting *DISC1* synthesis in mouse embryos with small interfering RNAs causes abnormal migration of neurons to the cerebral cortex.

More evidence comes from animal models developed in the past year. This fall, two Johns Hopkins groups, one led by Sawa and the other by Mikhail Pletnikov, published reports on two similar mouse models produced by introducing a truncated version of the *DISC1* gene into mice. Both lines showed similar changes. "The brain is superficially normal but isn't wired correctly," says Ross, a member of the Pletnikov team.

Outgrowth of neuronal projections called neurites, which help guide neuronal migrations, was reduced. In addition, interior brain spaces called ventricles were larger than normal—an alteration also seen in people with schizophrenia. And although it's not possible to diagnose mice as "schizophrenic," the animals showed certain behavioral changes seen in human patients, such as hyperactivity and social and cognitive impairment. (The Sawa team's results were published online 3 August in the *Proceedings of the National Academy of Sciences*; those of the Pletnikov team appeared online in *Molecular Psychiatry* on 11 September.)

In these mice, the mutant DISC1 protein exerted its effects in the embryos. Another Johns Hopkins group led by Hongjun Song has traced the gene's effects in the brains of adult mice. In these experiments, described in the 21 September issue of *Cell*, the researchers showed that inhibiting *DISC1* expression in newly formed adult brain neurons produces effects opposite to those seen in the other mouse models. Neurite outgrowth increased rather than decreased, and neurons migrated farther than normal. "If you disrupt *DISC1* function, everything goes faster," Song says.

Sawa notes that there are precedents for the same molecule having opposite effects depending on its context. Indeed, he is now working with Song and Pletnikov to identify the molecular change that can switch *DISC1*'s activity from inhibiting to stimulating neuronal migration. But whatever the outcome, researchers have long thought that schizophrenia is the result of aberrant brain development, and the results with these models buttress the case that irregularities in *DISC1* function contribute to that.



Brain disruption. Putting a mutant human *DISC1* gene in mice produces enlarged lateral ventricles similar to those of human schizophrenia patients.

CREDIT: PLETNIKOV ET AL., MOLECULAR PSYCHIATRY 1, 14 (2007)

A cAMP connection

Although it may not be possible to help patients by correcting abnormalities in brain development, work by Porteous and Millar, in collaboration with Miles Houslay of the University of Glasgow, U.K., suggests another tack to take. Two years ago, this group identified an enzyme called phosphodiesterase 4B (PDE4B) as one of *DISC1*'s many binding partners. This enzyme is a key regulator of cyclic adenosine monophosphate (cAMP), which transmits nerve signals into cellular responses, including those needed for memory formation.

The PDE4B enzyme breaks down cAMP after it has done its job in the cell, and further work by the Edinburgh group indicates that *DISC1* inhibits this activity until rising cAMP concentrations cause it to drop off the PDE4B molecule. Alterations in *DISC1* structure that disrupt the normal *DISC1*-PDE4B interaction might therefore interfere with learning and memory, among other things. "This is very important work," Sawa says. "Memory and cognition are both disturbed in schizophrenia and bipolar disease."

Additional evidence that disrupting the *DISC1*-PDE4B interaction can affect mental states comes from work on mouse models developed by Steven Clapcote and John Roder of Mount Sinai Hospital in Toronto, Canada, and their colleagues in collaboration with the Porteous team. (The results appeared on 3 May in *Neuron*.) By screening a library of mutant mice at the RIKEN research institute in Japan, the researchers identified two lines of mice, each with a different *DISC1* mutation that reduces *DISC1* binding to PDE4B.

Behavioral studies further showed that mice with one mutation display symptoms construed as schizophrenia-like, including hyperactivity and impaired learning and memory. Those symptoms were reduced by treatment with the drug rolipram, a PDE4B inhibitor, and also by treatment with two drugs used to treat human schizophrenia. The other mouse strain, Porteous says, had more depression-like symptoms. For example, when placed in water, the animals quickly gave up trying to escape and simply floated. These animals responded to treatment with antidepressant drugs. Developing drugs to regulate an enzyme such as PDE4B might lead to better ways of treating schizophrenia and other mental disorders.

The fact that *DISC1* associates with so many different proteins might help explain the diversity of conditions to which it has been linked. "It seems that *DISC1* acts as a scaffold around which other proteins cluster," Porteous says. Thus, the symptoms that develop in a given individual might depend on which interaction is altered by a genetic variation in *DISC1*. And conversely, variations in any of *DISC1*'s partners could also lead to abnormal brain development or function. Geneticists have begun hunting for linkages between these other proteins and various mental disorders.

Neurobiologists are heartened by what they've learned so far about *DISC1*. At the very least, the work has tapped into what could be a very important pathway for regulating brain neuron activities. As Ross puts it, "the findings support the idea that schizophrenia is a brain disease and can be studied the same way as degenerative diseases."

–JEAN MARX

LETTERS

edited by Jennifer Sills

The Origins of Human Bipedalism

THE REPORT BY S. K. S. THORPE *ET AL.* ON HAND-ASSISTED ARBOREAL bipedalism in orangutans certainly deserves attention ("Origin of human bipedalism as an adaptation for locomotion on flexible branches," 1 June, p. 1328). But does the discovery of orangutans engaging in human-like straight-leg bipedalism actually mean that "[h]uman bipedalism is … less an innovation than an exploitation of a locomotor behavior retained from the common great ape ancestor"? Although embraced by P. O'Higgins and S. Elton in their accompanying Perspective ("Walking on trees," 1 June, p. 1292), this interpretation embodies the Lamarckian use-disuse expectation that the postcranial features unique to humans and their fossil relatives would have emerged because a common human-ape ancestor had originally stood bipedally. But no known ape—fossil or extant—possesses the postcranial features associated with human-like bipedalism, and to anticipate that any number of years of early apes standing up in trees would have led to the developmental reorganization that underlies such profoundly human morphological novelty (*1*), while engaging the imagination, unduly stretches the bounds of biology.

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Reference

1. C. O. Lovejoy, M. J. Cohn, T. D. White, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 13247 (1999).

Response

SCHWARTZ IS INCORRECT TO CLAIM THAT OUR proposal is Lamarckian. We refer to selection eight times, and the main contribution of our paper is to identify a selective pressure that could have favored the adoption of upright, straight-legged bipedality in an arboreal context. That some of the postcranial features that facilitate such posture may be controlled by a limited number of developmental genes (*1*) is interesting, but unless Schwartz is proposing that natural selection cannot operate on such genes, it is not relevant to an assessment of our paper.

The most striking feature of modern human bipedalism compared with that of other vertebrates is that we walk with extended hips and knees (*2*), permitting substantial energy savings by exchange of potential and kinetic energies. In their facultative bipedalism, untrained captive orangutans and, as we show, wild orangutans (*3*) adopt trunk, hip, and knee postures much closer to those seen in human bipedalism than in untrained chimpanzees, bonobos, or gorillas. Even abnormally raised or trained chimpanzees that are habitually bipedal do not match the hip and knee extension seen in bipedalism of untrained orangutans (*3*). This strongly suggests that the anatomical features that permit erect, straight-legged bipedalism in orangutans, however controlled, have indeed been the subject of positive selection. These characteristics of orangutan bipedalism have almost certainly been selected for in an arboreal context, as part of a continuum of largely orthograde locomotor behaviors.

Because the common ancestor of crown hominoids is likely to have had a similar niche to orangutans—that is, to have been a ripe-fruit eater exploiting the peripheral canopy of tropical forest trees (*4*)—our findings are highly relevant to understanding the origins of human bipedalism. Features of the trunk and pelvis favoring upright walking were already present in early, arboreal, crown hominoids such as *Pierolapithecus* and *Hispanopithecus* (*Dryopithecus*) *laietanus* (*5*) [the latter also showing orangutan-like features of the hand (*6*)], and there is strong evidence for highly and habitually extended hips in the much later, partially or wholly arboreal crown hominoid *Orrorin* (*7*). These adaptations would certainly facilitate the adoption of habitual terrestrial bipedality by early hominins. Terrestrial bipedalism would then be expected to select for features of the hominin postcranium that enhance the effectiveness of human (terrestrial) bipedalism (*1*), e.g., adaptations limiting abduction of the thigh on the trunk (such as a short ilium) and a talocrural joint that favors parasagittal motion of the legs over the stance foot, also at the expense of abduction—as seen first in *Australopithecus anamensis* (*8*). Such selective forces eventually lead to the modern form of the human foot and pelvis, although this may not have been in place even in early *Homo*.

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5. C. V. Ward, in *Handbook of Paleoanthropology Vol. 2: Primate Evolution and Human Origins*, W. Henke, I. Tattersall, Eds. (Springer, Heidelberg, Germany, 2007), pp. 1011–1030.
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CREDIT: FRANKLINN VIOLA/ANIMALS ANIMALS

What's in a Name?

IF THE NAME LUPA FOR THE EUROPEAN DOG genome study was chosen after the Roman she-wolf ("Europe going to the dogs," E. Pennisi, News Focus, 21 September, p. 1670), the choice is not a felicitous one. The she-wolf legend was dismissed even by the Roman historian Titus Livius, who explained that the mother of Romulus and Remus was a certain Acca Laurentia, a very prosperous sex worker (to use a Dutch expression)—so prosperous that she left a lot of money to the city founded by her sons. In popular Latin, lupa meant she-wolf, but it also meant whore. Even today, in certain languages, we speak of brothels as lupanari (in Italian; the French have a similar word). Obviously, no one wants to have a whore on their standards, and that is how the she-wolf legend came about. I am afraid that our European colleagues made the same mistake as Mussolini, who called the preschool Italian children *Figli della Lupa*, thus sending a collective insult to Italian mothers. If the LUPA consortium were to change their name, I suggest JASPER, the name of my German shepherd, who is, of course, the best specimen of the best of all possible breeds.

RENATO BASERGA

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The Carbon Benefits of Fuels and Forests

THE POLICY FORUM "CARBON MITIGATION BY biofuels or by saving and restoring forests?" by R. Righelato and D. V. Spracklen (17 August, p. 902) provides limited perspective as a result of a single, relatively short time horizon and a limited consideration of the options available. Righelato and Spracklen conclude that the carbon sequestered by saving or restoring forest is greater than the emissions avoided by the use of the liquid biofuels. Although they may be correct given current technology, the case studies they analyzed, and a 30-year time horizon, their conclusion is dependent on site, technology, and time, and it does not apply to biomass used for direct combustion or gasification. Marland and Schlamadinger (*1*) showed that the carbon balance between restoring forests and producing biofuels is site-specific and depends on biomass productivity, the efficiency with which harvested material is used, the initial state of the surface vegetation, and the fossil fuel to be displaced. When forest products are used efficiently to displace carbon-intensive fossil fuels, and when productivity is high, sustainable harvest yields the greater carbon benefit, especially over a longer time period. Current-technology liquid biofuels represent low-efficiency conversion of harvest to energy, but direct combustion or gasification is more efficient at displacing carbon from fossil fuels. Righelato and Spracklen show that, over 30 years, even producing diesel fuel from woody biomass can begin to look "compatible" to reforesting temperate cropland. As we wrote in 1997, "there is not a one-size-fits-all strategy for optimal management of all land available for forest management to mitigate CO_2 emissions" (*1*). However, in many circumstances, biomass can produce greater carbon benefit than saving or restoring forests.

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Reference

1. G. Marland, B. Schlamadinger, *Biomass Bioenergy* **13**, 389 (1997).

Response

IN OUR POLICY FORUM (17 AUGUST, P. 902), we explicitly considered only liquid biofuels, which substitute for petrol and diesel. Large-scale replacement of fossil fuels in transportation is a more intransigent problem than the substitution of fossil carbon for heat and power considered by Marland and Schlamadinger (*1*), for which a range of carbon-free options exist, such as nuclear, wind, and solar power. We took a window of 30 years for our comparison of biofuels and forest restoration because this is the time scale that will likely be needed to develop and implement carbon-free transport-fuel technology. On this time scale, the current biofuels reduce carbon dioxide emissions less effectively than restoration of forests. As Marland, Obersteiner, and Schlamadinger indicate, there may be net carbon benefits from biofuels if longer time periods and new technology are considered. However, these avoided emissions would be too small and too late to meet targets of 60% or more reduction in emissions by 2050.

We noted in our Policy Forum that under some circumstances, fuel use of woody biomass may be compatible with retention of forest carbon stocks and may provide net carbon benefits similar to forest restoration in temperate zones. This is consistent with the model of Marland and Schlamadinger (*1*). However, land resources for arable substitution of transport fuels on the scale required are not available without further extensive deforestation, which would cause massive carbon dioxide emissions. Further demand for forest land to provide biomass for burning or gasification would need to be on a similarly large scale to meet emissions reductions targets. It is becoming increasingly clear that the risks associated with these land-use changes may outweigh any benefits. In our view, biofuels cannot provide a

CORRECTIONS AND CLARIFICATIONS

News of the Week: "CDC director's message on risk runs afoul of White House edits" by E. Kintisch (2 November, p. 726). The photo caption should not have said that White House science adviser John Marburger wanted to remove parts of proposed testimony by CDC Director Julie Gerberding on the public health effects of global warming. Marburger raised questions about portions of her testimony but did not suggest any cuts.

TECHNICAL COMMENT ABSTRACTS

COMMENT ON "Origin of Human Bipedalism As an Adaptation for Locomotion on Flexible Branches"

David R. Begun, Brian G. Richmond, David S. Strait

Thorpe *et al.* (Reports, 1 June 2007, p. 1328) concluded that human bipedalism evolved from a type of bipedal posture they observed in extant orangutans with seemingly human-like extended knees. However, humans share knuckle-walking characters with African apes that are absent in orangutans. These are most parsimoniously explained by positing a knuckle-walking precursor to human bipedalism.

Full text at www.sciencemag.org/cgi/content/full/318/5853/1066d

RESPONSE TO COMMENT ON "Origin of Human Bipedalism As an Adaptation for Locomotion on Flexible Branches"

Robin H. Crompton and Susannah K. S. Thorpe

Begun *et al.* purport to present technical concerns regarding our case for an arboreal origin for terrestrial bipedalism in early hominins, but merely reiterate their knuckle-walking hypothesis, which lacks support from the fossil record and is highly unparsimonious. The technical concerns are refuted by published studies cited in our study and thus do not affect our original conclusions.

Full text at www.sciencemag.org/cgi/content/full/318/5853/1066e

solution to our energy needs, but by appearing to be a "quick fix," they may distract us from developing effective, long-term, carbon-free solutions in the time window available to us.

RENTON RIGHELATO[1] AND DOMINICK V. SPRACKLEN[2]

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Reference

1 G. Marland, B. Schlamadinger, *Biomass Bioenergy* **13**, 389 (1997).

Eyeing a New Network

I. S. KOHANE AND HIS COLLEAGUES' POLICY Forum (11 May, p. 836) on "Reestablishing the researcher-patient compact" should evoke a response from clinical researchers. Healthcare organizations are keen to use large databases of information that have accumulated through record-keeping for clinical care delivery. Unfortunately, the consent process did not necessarily allow the freedom to contact patients as research subjects. The authors propose a prospective approach in developing informed cohorts, with linked medical and genomic information, to enable clinical research and the ability to recontact patients. We provide one example of how clinicians, their patients, and researchers can fully participate in and benefit from research.

In 2003, the National Eye Institute convened a broadly representative group to envision a National Genotyping Network for inherited eye diseases with two goals: to provide a resource for ophthalmic research in inherited ocular disorders, and to allow access to genotyping for patients and their doctors. A network of certified laboratories (*1*) was organized with a coordinating center to which a secure Web-based database was linked. In September 2006, the eyeGENE™ Network received its first sample (*2*). Phenotypic information was entered by the patient's doctor with the understanding that the patient and physician would receive a molecular genetic test result and that the physician would provide genetic counseling for that result. The anonymous DNA sample, with the linked genotypic and phenotypic data, was then placed in an open-source repository to enable future research. To date, the repository has 205 samples representing a diverse collection of heritable ocular conditions. The Network has not encountered any issues related to breaches in patient confidentiality or concerns about employment or insurance discrimination. This research project has been enthusiastically received by the ophthalmic and optometric community and stands as an example of how genomic research can be translated to patients.

IAN M. MACDONALD, BRIAN P. BROOKS, PAUL A. SIEVING

National Eye Institute, NIH, Bethesda, MD 20892–1860, USA.

References and Notes

1. Laboratories were certified according to the Clinical Laboratory Improvement Amendments (CLIA).
2. The National Ophthalmic Disease Genotyping Network, eyeGENE™ (www.nei.nih.gov/resources/eyegene.asp).

Letters to the Editor

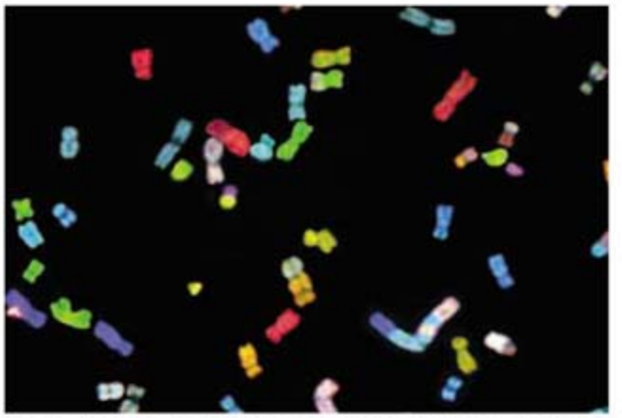
Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

MEDICINE

The Universal Darwinism of Disease

David C. Krakauer

Most remember the title of Darwin's revolutionary book, *On the Origin of Species by Means of Natural Selection*, but then forget the subtitle, *Or, the Preservation of Favoured Races in the Struggle for Life*. Whereas the former pertains to change and variation, the latter relates to stability and uniformity. The mathematical theory of evolution has sought to explore the consequences of mutation and variation on change and those constraints and evolved mechanisms that confer robustness on organisms. These robustness mechanisms are often dedicated to the prevention and elimination of disease.



Spectral karyotype. Multicolored painting probes reveal chromosomal rearrangements in an oral cancer cell.

Evolutionary theory is the right framework to adopt when we are seeking to account for the sources and constraints of variation in mutable lineages within large populations. This licenses, where appropriate, the application of Darwinism to a stunning range of phenomena, from the dynamics of genomes, the progression of disease, and the processes of speciation to the origin of language. In each case, we are dealing with random processes of variation, development, replication, drift, and selection. But the effectiveness of the theory, as for all powerful scientific theories, turns on the subtleties of quantitative rigor.

Dynamics of Cancer
Incidence, Inheritance, and Evolution

by Steven A. Frank

Princeton University Press, Princeton, NJ, 2007. 392 pp. $99.50, £59.95. ISBN 9780691133652. Paper, $39.50, £23.95. ISBN 9780691133669. Princeton Series in Evolutionary Biology.

In *The Dynamics of Cancer*, evolutionary biologist Steven Frank (University of California, Irvine) explores a theoretical immunology perspective on cancer—seeking to connect abundant data on coarse-grained phenotypic patterns to detailed microscopic, mutation-selection dynamics. The core macroscopic focus of the book is the age incidence curve of cancer, and the microscopic explanation resides in multistage progression.

Age-specific incidence records the number of cancer cases per year for a particular age group divided by the number of people in that age group. When representing age against incidence on a double logarithmic plot, one frequently observes a straight line that can be fit by a function of the form $I = ct^{(n-1)}$, where I is the incidence, t the age, and n the number of stages through which the cancer progresses. The constant c varies according to a number of different demographic and disease factors. Different cancers and different groups (males versus females, for example) tend to show very different patterns of incidence. Moreover, the slopes of these lines often depart from log-log linearity, and their age-specific gradients (acceleration) carry important information. It is these patterns—incidence and acceleration—that Frank seeks to explain, as he sees them as the primary, quantitative signatures of cancer.

The key to understanding incidence and acceleration is a multistage progression that describes a sequence of mutational events through which a tissue must transit on its path toward cancer. These mutations can be classified according to their impact on the balance of cell birth and cell death. Mutations in genes involved in programmed cell death abrogate the ability of cells to kill themselves when detecting damage. Mutations in tumor suppressors remove key constraints on cellular proliferation, and mutations in oncogenes typically stimulate cell division. Mutations in DNA repair pathways can lead to hypermutation and chromosomal instability, accelerating the rate of mutation toward cancer. Lastly, mutations in certain genes cause tumors to promote the growth of blood vessels required for tumor survival. The mutational spectrum is vast, but the underlying logic is often fairly simple. Important contributions of the book are the two theory chapters in which Frank develops a series of simple mutation-selection models. Building on pioneering work of Knudson, Armitage, Doll, and others, he aims to capture how variation in the sequential accumulation of mutations can generate the panoply of age incidence curves.

Frank's forte in the book is his search for the simplicity that is often masked by the complexities of cancer. With his mathematical models in hand, he turns to the details of cancer genetics, carcinogens, and aging and provides novel integrative insights. For example, his models identify potential causes for the slow age acceleration of melanoma versus the rapid acceleration of pancreatic cancer. Frank's parsimony-based approach to theory leads him to stress comparative analysis rather than curve-fitting. The comparisons follow a hypothetico-deductive model, whereby differences in progression are used to hypothesize differences in the age incidence curves of different cancers, e.g., why males tend to have more cancers early in life than females. Fitting typically seeks to match a family of models to a single body of data in order to infer the underlying dynamics. With a comparative (bottom-up) approach, there are fewer parameters and very dramatic differences to explain; with fitting (top-down), there is a high likelihood that the fit reveals little beyond the flexibility of the model assumptions.

A pervasive theme in the book concerns the lamentable, growing distance between molecular genetics and the kind of macroscopic theory Frank favors. As our measurement technology has become more precise and efficient, microscopic data enumeration has been emphasized over synthesis. This tendency is driven partly by expediency in the laboratory and partly by the absence of theory in the training of many molecular biologists. One of systems biology's avowed objectives is to unite coarse-grained mathematical and computational theory with microscopic laboratory data. Cancer has had a long history as a test bed for this kind of interdisciplinary approach.

Dynamics of Cancer emphasizes both the multiscale dynamics of the disease and an approach that synthesizes empirical knowledge with parsimonious, mathematical theory. Frank moves the field forward, narrowing the gap between a tragic disease of everyday life and the Darwinian world of the genome.

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10.1126/science.1149952

PHARMACEUTICALS

A Company with Great Impact

Anna E. Simmons

The history of Burroughs Wellcome and Company is synonymous with the development of a modern, research-based pharmaceutical industry in the United Kingdom. The firm was the first in the country to adopt many industry characteristics, such as the establishment of research laboratories and the use of detail men, and it was also a leader in product innovation and ethical advertising. In *Burroughs Wellcome & Co.*, historians Roy Church and Tilli Tansey provide a detailed, profusely illustrated account of the company's first 60 years.

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Burroughs Wellcome was established in London in 1880 by two American pharmacists, Silas Mainville Burroughs and Henry Solomon Wellcome. Both brought key skills to the partnership: Burroughs contributed initial capital and innovation, foundations that Wellcome then built on. The firm soon expanded its business overseas and introduced detail men to market its products directly to medical practitioners, chemists, and druggists. In its first decade, the company underwent a transition from trading to manufacture, which culminated in the 1889 opening of its main factory in Dartford, Kent, and established itself as a leader in the British pharmaceutical industry.

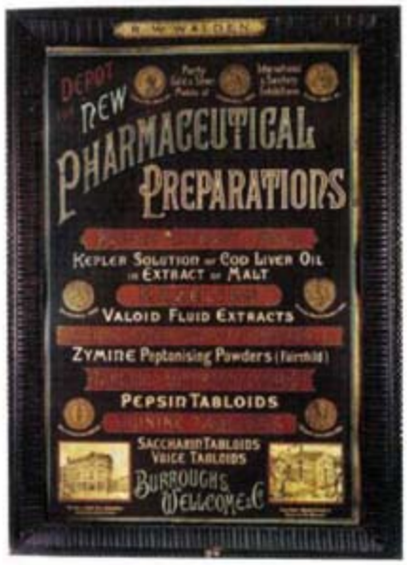
Burroughs Wellcome & Co.
Knowledge, Trust, Profit and the Transformation of the British Pharmaceutical Industry, 1880–1940

by Roy Church and E. M. Tansey

Crucible Books, Lancaster, UK, 2007. 592 pp. Paper, $39.99, £19.99. ISBN 9781905472079.

The themes of knowledge, trust, and profit are central to understanding the firm's development. Trust provides a particularly interesting perspective, highlighting both strengths and weaknesses in the business operation. Despite their many achievements, Burroughs and Wellcome distrusted one another to the extent that after various disagreements, Burroughs sought to terminate the partnership. Wellcome triumphed in the bitter legal battle that followed, but the dispute was still continuing in 1895 when Burroughs suddenly died in Monte Carlo from pneumonia. Later on, an absence of trust between key staff members would cause difficulties during the interwar period.

However, these personnel problems did not affect customers' trust in the firm. A reputation for high-quality products and ethical advertising was central to its relations with both physicians and pharmacists. This trustworthy reputation was enhanced by Henry Wellcome's objective to advance medical knowledge through the creation of research laboratories, whose scientists could publish freely and pursue independent lines of research. Such an ethos undoubtedly assisted Henry Wellcome's eventual success in 1901 in registering the Wellcome Physiological Research Laboratories under the 1876 Cruelty to Animals Act. That achievement—which the authors describe as "possibly



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the most important contribution Wellcome made to medical research in Britain"—allowed the firm to carry out the animal testing required for the production of antitoxins and set a precedent for the registration of other laboratories associated with commercial manufacturers.

While recognizing the worth of trust and knowledge in pharmaceutical manufacturing, Henry Wellcome also understood commercial demands, and he emphasized the importance of "quality for profit." In the years leading up to the First World War, net profits grew steadily to just over £80,000. This growth did not continue indefinitely, and average annual profits declined sharply between the 1920s and 1930s. Placing the business in the context of wider economic and industrial trends and examining the minutiae of the firm's administration and finances, Church and Tansey provide a more accurate interpretation of the problems Burroughs Wellcome encountered than have previous histories. Holding back from directly appealing to consumers through the mass media and remaining loyal to individual retail chemists (rather than discounting prices for multiple-outlet wholesalers such as Boots), the company was left behind by crucial developments in pharmaceutical retailing. Despite its history of research excellence, it launched only one important innovative product (Digoxin, a substitute for digitalis) during the 1930s, while it also failed to diversify and develop new lines of general goods. Knowledge, trust, and profit, the authors argue, are also central to understanding the difficulties the firm faced.

The legacy of Burroughs and Wellcome stretches well beyond the firm they created and the time span covered in the book. Many who trained in the Wellcome laboratories later carried out pharmaceutical and medical research in academia, government, and industry. The caliber of the researchers is striking: the authors highlight 17 Wellcome scientists who became fellows of the Royal Society and one Nobel laureate, Henry Dale. Such individuals also shaped the growth of a research and development–based pharmaceutical industry in Britain, as Burroughs Wellcome inadvertently supplied many of its rivals with ready-trained scientific staff. By 1940, Boots, May & Baker, Glaxo, and British Drug Houses all employed research directors who had previously worked for Burroughs Wellcome. These influences have continued. The Wellcome Trust is the largest private funder of medical research in Britain and, with the recent opening of the Wellcome Collection in London, brings Henry Wellcome's legacy to a much wider audience. (The Trust also provided "generous funding support" for the book.) Much more than a company history, this attractively presented and comprehensively researched book shows how one firm transformed the pharmaceutical industry in Britain.

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PUBLIC HEALTH

Biobanks in Developing Countries: Needs and Feasibility

Technological advances coupled with use of existing resources can be used to create biological repositories that may lead to better health in developing countries.

S. K. Sgaier,[1]* P. Jha,[1]† P. Mony,[1,2] A. Kurpad,[2] V. Lakshmi,[3] R. Kumar,[4] N. K. Ganguly[5]

Over 90% of the global burden of disease is in developing countries, yet only 10% of global research addresses many of these diseases. Between 1975 and 1999, ~1% of new marketed drugs were for tropical diseases and tuberculosis (*1*). Repositories of biological samples linked with medical data from individuals (biobanks) are infrastructures for sustained research on the biological determinants of disease and promise to accelerate the discovery of vaccines, drugs, and diagnostics. However, the distribution and focus of current biobanks suggests that their discoveries will not sufficiently benefit those living in developing countries. Innovative use of recent technological advances and existing infrastructure platforms make biobanks cost-effective and feasible in developing countries.

Biobanks
(with sample sizes of ≥200,000 or in a developing country)

Name of biobank	Size (age group)	Start-up cost* (cost per person)	Chronic disease	Infectious disease
U.K. Biobank	500,000+ (40–69 yr)	$120 million (~$240)	Yes	No
Estonian Genome Project	~ 1 million†	$2.5 million‡ (~$250)	Yes	No
Icelandic deCode Biobank§	~250,000	$212 milion (~$850)	Yes	No
Kadoorie Study of Chronic Diseases in China	500,000 (35–74 yr)	$22 million (~$50)	Yes	Some
The Mexico City Prospective Study	160,000 (35+ yr)	Not available	Yes	Some
The Gambian National DNA Bank	~57,000	$0.6 million‖ (~$15)	No	Yes¶
The Indian National Biobank§,#	~2–3 million (18+ yr)	$20–$30 million (~$10)	Yes	Yes

*Estimated, in U.S. dollars. †This is 75% of the Estonian population. ‡For a pilot project of 10,000 participants. §Familial linkage studies are possible for these two biobanks, and genetic association studies are possible for all biobanks listed. ‖For the first 5 years. ¶Powered to discover correlates of nonfatal TB and/or malaria and not HIV/AIDS, which has low prevalence in The Gambia. #Presently at the design stage.

Biobanks as a Platform

The Human Genome Project, annotation of millions of single-nucleotide polymorphisms (SNPs) within the genome, development of ultrahigh-throughput genotyping, small-molecule detection methods, and powerful software to analyze the mass of data that is generated, now make possible the discovery of the allelic and biological variants that underlie complex diseases (such as cardiovascular diseases, cancer, diabetes, tuberculosis, and AIDS). Common genetic variants likely involve moderate effects, such as a relative risk of 1.2. Reliable assessment of these variants in different populations (including documenting any interactions between genes and other risk factors) requires studies with thousands, or even tens of thousands, of cases and controls.

A few biobanks have already been established in developing countries such as the Chinese Kadoorie study (*2*), the Mexican biobank (*3*) and the Gambian national DNA bank (*4*) (see table, above). The Chinese and Mexican biobanks were designed primarily to discover correlates of noncommunicable diseases in adults over age 35 years, and the Gambian study is relatively small. These studies are not sufficiently representative of the major causes of death and disability in developing countries or of the age groups at which disease strikes. In particular, HIV/AIDS, tuberculosis, and malaria require larger studies in diverse populations.

Disease Burdens in Developing Countries

More than 75% of the 5 million deaths worldwide due to HIV/AIDS, tuberculosis, or malaria are in developing countries. Even infectious diseases have cofactors, which make their acquisition or conversion to clinical disease more likely, such as smoking and tuberculosis (*5*).

Genetic or undiscovered copathogens may help explain the unprecedented increases in HIV-1 in eastern and southern Africa. Natural resistance to HIV-1 appears to be evolving among select populations (*6*) that are constantly being challenged by the virus. Understanding how the immune systems have so evolved to fight these infections can enable new drugs and vaccines. Focused biological research on the genetic and other biological correlates of infectious diseases is under way, most notably by the Grand Challenges in Global Health (*7*). These need to be complemented by more open-ended platforms for unpredictable discoveries, as is possible with larger biological repositories linked to medical data.

Developing countries, unlike most developed countries, suffer from the dual burdens of chronic (chiefly noncommunicable) diseases and infectious diseases (*8*). Already, four out of five chronic disease deaths occur in developing countries. The genetic and environmental variations that contribute to complex chronic diseases are not necessarily the same in geographically segregated populations. Indeed, common chronic diseases can have surprising correlates. A 10-year prospective study in China, for example, found higher risks of vascular deaths among people with excessively low, as well as those with excessively high, body mass even after adjusting for smoking and blood pressure (*9*). Two small studies in India (*10*) and in Iraq (*11*) found that low body mass and diabetes were correlated with tuberculosis history and a positive tuberculin test, respectively.

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A Way Forward

Typical biobanks are expensive partially because the serum samples that they collect and keep must be kept cold continuously. Dried blood spots (DBSs), in contrast, do not require refrigeration during collection or transport (*12*). DBS samples can be easily collected and safely transported by regular mail. The higher acceptability by participants of DBS versus whole-blood collection, lower costs, and ease of handling also enable much larger sample sizes to be achieved within a given budget.

MDS health surveyor collecting a DBS sample in the field.

CREDIT: SEMA K. SGAIER

Despite their small volume, DBS samples have been increasingly used for molecular, enzymatic, immunological, biochemical, and hematopoietic analyses. SNPs within the whole genome can now be reliably assessed from DBSs (*13*). The continuous decline in the sample volume and the cost of SNPs and the development of high-throughput analyses, mean that biobanks based on DBSs are becoming economical, as well as scientifically practicable. The major roadblock is getting reliable epidemiological evidence about the relevance of variables measured to the development of disease. Appropriately large-scale epidemiological fieldwork in developing countries to acquire blood samples systematically linked to relevant measures of disability and future mortality is crucial. Biobanks in Western countries use their national health systems, with physicians collecting samples and medical data for their patients. In developing countries, however, fewer people have access to medical care, and linkage to routine health care is not yet possible.

Several developing countries have established disease and mortality surveillance systems, which could be cost-effective platforms for biobanks. A good example is the antenatal clinic HIV surveillance system recommended by the World Health Organization to track changes in HIV prevalence among pregnant women in 132 countries. This widely implemented system is not used adequately to understand the transmission and correlates of HIV infection, primarily because collected samples are usually discarded after HIV testing. Modest enhancement of this system with additional demographic and medical information, as well as reliable archiving of samples, would provide a widely practicable resource to investigate the biological correlates of HIV. Other examples of established surveillance systems include surveys of malaria parasites and the INDEPTH network of 37 demographic surveillance sites—26 of which are in Africa (*14*).

India: A Case Study

Over the past 30 years, the Indian government has built a population-monitoring framework called the Sample Registration System—a nationally representative sample of 7.6 million people in 1.3 million households across the country. The "Million Death Study" (MDS) (*15*) is under way within this system. The Indian Council of Medical Research, Registrar General of India, and the University of Toronto are collaborating to explore the logistics of building a national Indian biobank to assess the underlying risk factors and correlates of disease in India. The MDS is unique because it collects data that will allow both family-based genetic studies and case-control association studies. The design and scale should ensure that for each complex disease of interest, several thousand cases and controls (efficiently tested through "nested" case-control design) will be available for association studies with sufficient statistical power to detect modest but medically relevant associations.

We are exploring methodological and logistical issues of building a national biobank in India. Medical and family history, blood pressure, body mass, smoking and alcohol use, other variables, and DBSs were collected in household surveys in 2006 from 2700 adults aged 18 years or older in six districts in three states (see figure, left). Only 5% of the people interviewed refused to give DBSs, in contrast to refusal rates of nearly 40% in a similar study collecting a serum sample (*16*). The likely costs of a DBS-based Indian biobank would be at least 1/20th the cost per person of the ongoing serum-based U.K. Biobank (see table, p. 1074).

Toward a Global Consortium of Biobanks

National or regional biobanks are a first step. Others have called for a global consortium of biobanks to address common ethical issues, data ownership, and data sharing (*17*, *18*). We see these consortia arising for two reasons. First, joint analyses of important, but uncommon, gene variants will be needed to generate more definitive results than can be generated from individual (and likely underpowered) studies. Second, reasonable expectations from funders and beneficiaries will push toward collaboration, as has happened with the Human Genome Project (*19*) and the Global HIV Vaccine Enterprise (*20*). The promise is enormous: accessible and affordable studies in diverse populations to permit imaginative search for common and rare genetic and other biological correlates of global diseases.

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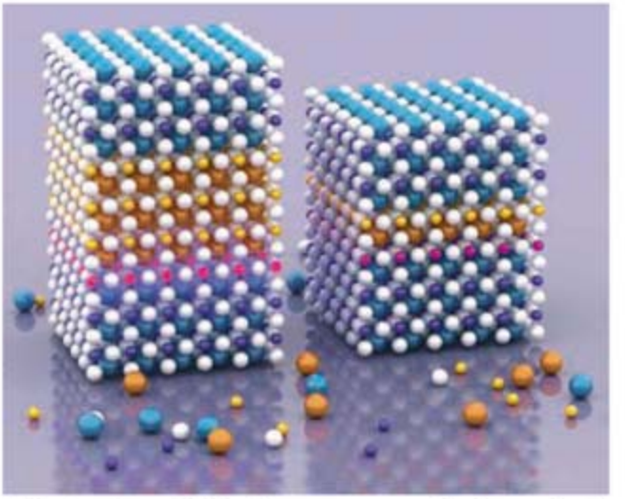
PHYSICS

When Oxides Meet Face to Face

New devices may be possible once we understand the interface between oxide materials such as superconductors and ferromagnets.

Elbio Dagotto

Multilayer structures have emerged as a leading research topic now that atomically precise methods for preparing them are available. In particular, researchers expect that oxide multilayers may lead to interesting artificial materials with novel properties (see the figure). Several of the oxides used in these multilayer systems belong to a family of compounds with strongly correlated electrons. The exotic properties of these oxides include high–transition temperature (T_c) superconductivity, with critical temperatures far higher than in standard superconductors, and colossal magnetoresistance (CMR), where the application of magnetic fields of a few teslas changes the resistivity by orders of magnitude. On page 1114 of this issue, Chakhalian *et al.* (*1*) report x-ray analysis and calculations of the interface between two such oxides.



Oxide interfaces. Models of heterostructures of lanthanum aluminate between strontium titanate layers. The atoms are represented by colored spheres (oxygen, white; lanthanum, orange; aluminum, yellow; strontium, large blue; and titanium, small dark blue). Although the materials alone are insulating, the electrical conductivity of the bottom interface (purple spheres, brighter on the left indicating higher conductivity) can be tuned due to coupling with the top interface (*11*).

These authors prepared a heterostructure with layers of (Y,Ca)$Ba_2Cu_3O_7$ (YBCO) and $La_{2/3}Ca_{1/3}MnO_3$ (LCMO) and observed a transfer of charge from the Mn oxide to the Cu oxide. This induces important modifications in the electronic orbitals of the atoms at the interface. For example, the orbitals designated $d_{3z^2-r^2}$ (*2*) are considered irrelevant in bulk cuprates because they are fully occupied and cannot act as bridges for current flow. At the interface, however, they become partially occupied and can move electrons between the two oxides. The interfacial orbitals undergo "reconstruction"—that is, they are deformed from their normal shape so that the electronic structures of the two different oxides can blend. Most of the oxides under consideration for heterostructures are very sensitive to reconstruction, something that must be considered in all future designs of artificial oxide multilayers.

High-T_c superconductivity and CMR are examples of "emergent" phenomena—new properties that cannot be anticipated from the local interactions among the electrons, and between electrons and the lattice. Many recent investigations have revealed the complex nature of these materials in bulk. These hard ceramic materials seem to hide a "soft" electronic component that produces nonlinear responses to small perturbations, as well as emergent behavior (*3*, *4*).

Artificial thin-film oxide structures could thus make the already complex individual properties of bulk strongly correlated oxides even more interesting. The oxides used in these structures could have different bulk properties, but they all have similar lattice constants (i.e., distances between atoms), allowing for a good match at the interfaces. The number of combinations of these oxides is enormous, and the potential for novel behavior is a strong motivation for these investigations.

If interfaces of semiconductors—which are rather featureless materials with a nonmagnetic rigid lattice—can nonetheless lead to fascinating physics such as the quantum Hall effect, imagine what could be done with oxides. A variety of exotic two-dimensional electronic systems could be stabilized at the oxide interfaces, exploiting spin, charge, and orbital interactions as well as lattice vibrations. Recent investigations have already shown that both a metal (*5*) and a superconductor (*6*) can be induced at the interface of two insulating materials, and the number of surprises will surely continue to grow.

Charge transfer at oxide interfaces (*1*, *7*) produces novel two-dimensional phases, as well as charge doping without the typical disorder caused by chemical doping. Thus, interfaces provide an interesting venue for doping oxide perovskites (such as the high-T_c materials) with carriers (*8*). Technological applications are also possible. For example, several groups are working on compositionally graded interfaces made from manganites with the hope of achieving high-performance magnetic tunneling junctions (*9*).

The field of "oxide electronics" is growing fast (*10*), although achieving the mobility levels and purity seen in semiconductor heterostructures remains a challenge. The combination of all these issues is what makes oxide heterostructures so interesting: This area of research is located at the intersection between fundamental science investigations and technological applications.

Previous studies of oxide heterostructures were framed in terms of "lattice reconstruction." That is, because interfacial ions are subject to forces different from those in bulk, these atoms can change position. And the mere transfer of charge at interfaces can lead to "electronic reconstruction" (*7*, *11*). In this case, the different electronic density at the interface relative to the bulk is the origin of the exotic properties. Chakhalian *et al.* have now introduced orbital reconstruction as a third process. Although the $d_{3z^2-r^2}$ orbital is widely thought to be unimportant in bulk cuprates, it is known to be crucial in bulk manganites. As cluster calculations suggest (*1*), the reported strong Cu-O-Mn bond that leads to the orbital reconstruction is precisely caused by the $d_{3z^2-r^2}$ orbital becoming active on both sides of the interface.

Despite recent activity, the field of oxide interfaces remains virtually unexplored. What might happen if we could mix materials with vastly different properties such as ferromagnets, antiferromagnets, superconductors, ferroelectrics, multiferroics, geometrically frustrated spin systems, heavy fermions, and others? Considering this enormous number of combinations, theoretical guidance is needed. But for theory to be useful, the calculations must be reliable, at least qualitatively. Powerful techniques are needed to study inter-

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faces of strongly correlated electronic systems. Reliable procedures to calculate and measure work functions of individual materials are also needed to predict the direction of charge transfer at interfaces. The collective responses and nonlinearities of models for oxide interfaces must be carefully analyzed. By this multilevel effort, the potential new functionalities and exotic phases of the oxide combinations under scrutiny will hopefully be revealed. Lattice, electronic, and now orbital reconstructions will all be essential in the effort to understand and use oxide interfaces.

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GENETICS

Widespread Monoallelic Expression

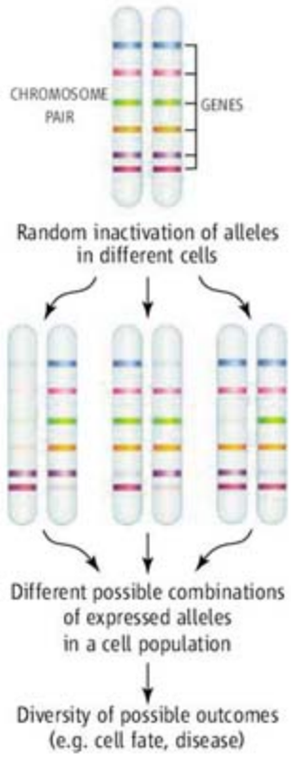
The surprisingly high prevalence of random allele inactivation in human cells can generate diversity in gene expression that affects cell fate and physiology.

Rolf Ohlsson

Most eukaryotic cells have two copies of autosomal (non–sex-determining) chromosomes. Although both copies (alleles) of individual genes are usually expressed in each diploid cell, one of the alleles is inactivated in a subset of genes (*1*). It is of profound interest that monoallelic expression in somatic cells does not simply represent a rheostat control for gene expression. Rather, it often operates in some selective function, such as determining the repertoire of odorant receptors or T cell receptors that are expressed (*2*, *3*). Moreover, the parental alleles of some mouse genes, such as those that encode cytokines, are expressed in random patterns, in which either, neither, or both alleles are inactivated, potentially influencing the selection and expansion of particular on T cells (*4*).

On page 1136 in this issue, Gimelbrant et al. (*5*) report that the mammalian genome employs random, monoallelic expression more extensively than thought. This may be to generate diversity in expression patterns on an unprecedented scale, which has important implications for the ontogeny of human diseases.

Gimelbrant *et al*. determined the proportion of human genes that can be expressed monoallelically, in patterns that are epigenetically stable (for example, chemical modifications of DNA, such as cytosine methylation, that do not alter the sequence but are heritable within cell populations). They identified expressed alleles in cloned cell populations of human B lymphocytes by taking advantage of polymorphic sequences, or single-nucleotide polymorphisms (SNPs). By modifying a method for detecting SNPs in DNA sequences, Gimelbrant *et al*. were able to track expressed messenger RNA (mRNA) sequences. Because most of the SNPs for the 3939 genes that were assessed are located in noncoding introns that are not present in mature mRNA, the authors included precursor forms of mRNAs (which contain introns) in the samples that were analyzed. They identified 371 genes (nearly 10%) as monoallelically expressed in epigenetically stable patterns in at least one population of cells derived from a single B cell clone. Although most of these genes were also found to be biallelically expressed in some other B cell populations, up to 20% could be consistently expressed from one of the parental alleles in some B cell clones. Thus, each cell population displays a vast heterogeneity in patterns of mono- and biallelic gene expression, providing numerous combinatorial patterns of gene expression (see the figure).



Generating diversity. Alleles are randomly inactivated on a pair of chromosomes in a human somatic cell. The various patterns of inactivation in progeny cells are then stabilized (epigenetically). This can generate diverse cellular and physiological outcomes.

Although other patterns of monoallelic expression have been described in human cells (*6*, *7*), Gimelbrant *et al*. go much further in two important respects. First, they analyze a large number of genes, thereby increasing the generality of their conclusions. Indeed, based on their findings, the authors argue that more than 1000 genes in the human genome can potentially be monoallelically expressed at any given time, indicating an amazing degree of diversity in possible combinations of expressed alleles. Interestingly, genes encoding cell surface receptors are overrepresented in this subset, suggesting the enormous potential for epigenetic regulation of receptor-mediated cell-cell communications, and hence, the regulation of cell diversity and cell fate (*8*). Second, Gimelbrant *et al*. hint at the fascinating possibility that the same set of genes can be monoallelically expressed at certain stages of development in a subset of tissue cells and/or perhaps in only some individuals.

It is unclear, though, how random monoallelic expression is established in somatic cells. Unlike the acquisition of imprinted, methylated marks on DNA from the parental germ lines–so-called genomic imprinting, which inactivates either a maternal or paternal allele during gamete development (*1*)—random inactivation of nonimprinted autosomal alleles occurs in somatic cells. Gimelbrant *et al*. show that many of the monoallelically expressed genes are highly expressed, apparently ruling

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out concerns about stochastic monoallelic transcription events postulated to occur at low levels of transcription (*1*). Perhaps both alleles are in different transcriptional environments within the nucleus. Indeed, alleles that display asynchronous replication, a hallmark of many monoallelically expressed genes (*2*), can occupy different positions within the nucleus (*9*). It may also be that limiting amounts of protein complexes that remodel chromatin (the DNA and protein constituents of chromosomes) could randomly inactivate alleles. Finally, differentially modified (methylated) DNA regions that are adjacent, far apart, or even on different chromosomes might interact and modulate allele-specific epigenetic states and transcription (*10*). Such possible explanations can now be tested.

The data from Gimelbrant *et al.* could fundamentally influence current views on the mechanisms of some pathological conditions such as haploinsufficiency (having only one functional allele, which produces insufficient amounts of product) or the loss of function of tumor suppressor genes. In both cases, the loss of one allele might lead to a dramatic effect if the remaining allele already carries epigenetic marks that render it inactive (*11*). Consequently, anyone unfortunate enough to possess the "wrong" set of monoallelically expressed genes might be susceptible to the earlier onset of a complex disease, such as Alzheimer's disease. The interplay among genotype, epigenotype, and gene inactivation will now become more important for understanding developmental mechanisms, penetrance of diseases, and responses to medical treatments in an individual.

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MATERIALS SCIENCE

DNA Circuits Get Up to Speed

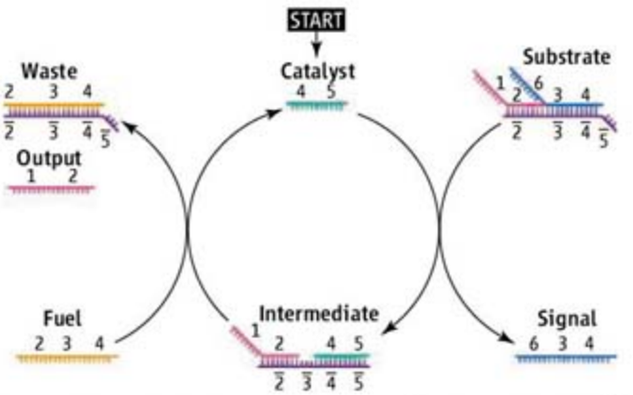
An amplification mechanism brings DNA circuits closer to practical applications.

Roy Bar-Ziv

In the past three decades, scientists have shown that DNA can be programmed into higher-order structures and used to encode mathematical computation (*1*–*3*). Many attempts have been made to create DNA-based devices and machines of a mechanical and computational nature (*3*–*5*). Yet DNA nanotechnologists still face substantial challenges in passing from proof-of-principle demonstrations to functional devices that are reliable, quick, and extendable. On page 1121 of this issue, Zhang *et al.* (*6*) report an important step toward this goal.

In contrast to solid-state devices, DNA-based systems operate at the nanometer scale in an aqueous environment, where thermal motions render molecular interactions imprecise. Hence, in seeking programmable biochemical functions, scientists must invent designs that are hierarchical and resilient to spurious interactions and signal degradation.

Living organisms rely on genetic biochemical networks to regulate complex functions such as sensing, adaptation, timing, and—ultimately—reproduction. These networks have evolved in noisy environments and are hence inherently robust, often endowed with high-fidelity capabilities. They are composed of genes and their respective RNA and protein products and exhibit a variety of dynamic patterns. Key players in these biochemical functions are enzymes that can pick up even faint chemical signals, amplify their meaning for the organism, and relay them rapidly for subsequent processing.



The catalytic cycle of Zhang *et al.* The catalyst's **5** domain binds the $\bar{5}$ toehold of the substrate, triggering a rapid branch migration process that leads to the intermediate complex and the release of the signal strand. Subsequently, the **3** domain of the fuel binds the intermediate's $\bar{3}$, inducing two branch migrations and releasing the output strand, a waste complex, and the catalyst for another turnover.

Artificial biochemical circuitry may one day play an analogous role in human-made systems. Imagine circuits that control DNA-based motors and sensors, similar to electric circuits controlling electromechanical devices. For example, a recent report described DNA circuits (*4*), in which the input and output signals, as well as the logic gates, were represented by distinct DNA strands—all in a test tube. Computations were performed by hooking up and cascading gates and signals in a manner similar to electric circuits. However, adding more components incurred long response time and signal loss, thereby forming a bottleneck for nontrivial computation. Hence, a fast and modular amplification mechanism is essential.

Zhang *et al.* now propose an elegant solution to this signal amplification bottleneck. In essence, they emulate the concept of the biochemical enzyme, but with DNA strands only. The mechanism alters no covalent bonds and is driven by entropy (rather than energy, as in most biological catalysis). The design is based on the separation of binding energy scales, making it easy to understand, robust, modular, and potentially useful.

The basic species are six short, single-stranded DNA domains that interact via Watson-Crick base pairing. Strands composed of these domains make up all the players in the reaction: substrate, fuel, output signals, catalyst, and waste (see the figure). The core domains of the substrate (domains $\bar{2}$, $\bar{3}$,

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and $\bar{4}$) are paired to the signal and output strands, whose domains **1** and **6** are unpaired, as is the substrate's dangling end domain, $\bar{5}$.

The goal is to release the signal and output strands. Naïvely, in the absence of a catalyst, this can be done solely by the fuel—a strand composed of domains **2**, **3**, and **4**—which replaces the two strands. The signal and output strands are then released to solution along with a waste by-product composed of the substrate core and the fuel. In this catalyst-free exchange reaction, the same domains are base-paired in the initial and final states, so there is no net change of binding energy. However, the overall configurational entropy increases in passing from two to three free molecules. The reaction is thus thermodynamically favorable. Yet a large kinetic barrier is incurred, because the catalyst-free exchange reaction must proceed by unzipping the substrate one base after another—a highly improbable sequence of events that would take a very long time.

To push forward the reaction, Zhang *et al.* introduce a catalyst composed of domains **4** and **5**. The catalyst's domain **5** can now pair with the substrate's domain $\bar{5}$ to trigger the rapid displacement of the signal by a process called branch migration. The catalyst thus reduces the kinetic barrier and stabilizes an intermediate complex (see the figure). The fuel matches the exposed domain **3** of the intermediate and, using the same branch migration trick, displaces the output while irreversibly sealing a waste duplex and ejecting the catalyst back to solution for another turnover. This catalytic cycle can accelerate the initial reaction by four orders of magnitude.

Zhang *et al.* demonstrate two applications of amplification using their catalytic scheme. First, a two-stage cascade was constructed by introducing an upstream catalyst system whose output acts as the catalyst to another system. The circuit amplifies the initial catalyst by a factor of 900. Second, an autocatalytic circuit was constructed by encoding the catalyst as a part of the output, such that reaction closes a loop on itself, displaying exponential growth kinetics.

Why is the catalytic reaction useful? Embedded in a biochemical logic circuit, it can be used to amplify weak signals and thus increase the speed and performance of the circuit. To construct multicomponent circuits, unwanted cross-talk between components must be avoided. Zhang *et al.* achieve this by design, because complements of the long, strongly binding "specificity" domains (**1**, **2**, **4**, and **6**) never appear in single-stranded form. Molecules can interact only via the short, weakly binding "toehold" domains (**3** and **5**). This separation of energy scales renders the design extendable and tractable.

Catalytic DNA circuits of the kind described by Zhang *et al.* could be coupled to various biochemical reactions to analyze heterogeneous macromolecular mixtures, detect weak biochemical signals, and control nanoscale devices. Combining such enzyme-free DNA circuits with biosynthetic reactions may get us closer to understanding nature's design principles. DNA circuits could also be integrated into materials platforms, such as gels, polymers, and surfaces, to generate active programmable systems (*4*, *7*–*10*).

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CANCER

The Gene Topography of Cancer

Despite debates over technologies and statistics, a new catalog of genes associated with colon and breast cancer is close at hand.

Jeffrey M. Trent and Jeffrey W. Touchman

There are three key questions any cancer patient asks when confronted by his or her diagnosis: What type of cancer do I have? What is my outlook? What can be done about it? Increasingly, the answers are coming from DNA-based sequence information. Decades ago, scientists discovered that defective genes can cause cancer (*1*, *2*), thereby establishing a paradigm for tumor biology and providing an important motivation for embarking on the Human Genome Project. Yet this remarkable sequencing achievement marked only the beginning of the quest to fully understand the biology of cancer. With the complete sequence of nucleotide bases in normal human DNA available, scientists now must classify the wide array of human genes according to their involvement in tumorigenesis. On page 1108 of this issue, Wood *et al.* (*3*) advance this endeavor by describing the mutational spectrum of nearly every well-annotated human gene [18,191 distinct genes from the Reference Sequence (RefSeq) resource] in 11 breast and 11 colorectal tumors through systematic sequencing of coding exons.

The resulting sequence "landscape" of these tumor genomes is striking. The authors find a scattering of five well-studied genes that are commonly mutated (which they call "mountains"), as well over 200 genes mutated at a lower frequency ("hills") in these two cancers. For the first time, there is evidence that most of the mutations that drive cancer may not occur in such gene mountains, but rather are spread across heterogeneous gene hills.

These findings are generating some interesting opinions. Viewpoints differ as to the value and timeliness of using current established technologies (e.g., polymerase chain reaction and Sanger sequencing) to define the mutational landscape of cancer, versus waiting for the "certain" promise of next-generation, whole-genome sequencing approaches. In the 1990s, the argument for waiting for technology improvement was one of expense and throughput as well as the limited expectations of identifying medically useful information. To some extent, that is the argument again today as new technologies will likely be less expensive, provide haploid resolution (that is, differentiate the maternal from the paternal contribution to the genome), and hold the possibility of much more complete genome-wide sequence coverage. Even granting that technological progress in genomic sequencing is at hand, the question remains how much more we will see. What is clear is that by using existing technology, anchored to expert insights into cancer biology and clinical therapeutics, Wood *et al.* have revealed a previously unrecognized view of the genetic landscape of human cancer.

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Scanning for genetic mutations in human cancer genomes. Traditional automated approaches to DNA sequencing are yielding "mountains" of commonly mutated cancer-causing genes, as well as many of the less frequently mutated genes, found in "hills." Cost reduction and new technology innovations will be required to analyze the genomes of the hundreds of known tumor types.

Unquestionably, a variety of thorny statistical issues are involved in the analysis of data obtained by the authors. Are the candidate cancer genes mutated at rates higher than the experimentally determined "passenger" mutation rates (passenger mutations provide no positive or negative selective advantage to the tumor, but are retained by chance during repeated rounds of cell division and clonal expansion)? How does the passenger mutation rate affect the discovery of candidate cancer genes? How does variation in passenger mutation rates among genes affect the analysis, given that the rates of background sequence variation can exhibit heterogeneity across the genome (*4*)?

These challenging issues have been nicely addressed by the Empirical Bayes approach described in Wood *et al.* (*3*). They describe "passenger probabilities" for each candidate cancer gene, which provides estimates of the likelihood that the observed mutations occurred by chance. These take into account the precise nucleotide composition of each gene, the mutation spectrum of the tumor type, and estimates of the background mutation rate. Yet, despite our powerful ability to identify genes that are mutated at unusually high rates, generally one cannot determine with absolute certainty whether the higher rate is the result of higher intrinsic mutability or of positive selection during tumorigenesis. What we hope to accomplish with DNA sequence data from many tumor samples is to select genes for more in-depth functional analysis on the basis of their mutation characteristics and frequency.

What are the prospects for translating the discovery of new cancer genome mutations into treatments for cancer? Sequencing is being used to diagnose a specific cancer subtype, to help match a given patient with a particular treatment, and to monitor therapeutic response—so-called personalized medicine (*5*–*7*). Although new molecular biomarkers (DNA-based or otherwise) are not yet available for all cancers or subtypes, there are now multiple examples that have directly proven the power of precise nucleotide-centered diagnosis to better manage disease. The performance of new gene-based therapeutics is encouraging, such as in chronic myelogenous leukemia (Gleevec), some forms of breast cancer (Herceptin), and some lung cancers (Iressa and Tarceva), although the list of such interventions remains far shorter than it would be if researchers in academia and the private sector had ready access to the entire spectrum of genomic changes that occur in cancer (a fundamental basis for The Cancer Genome Atlas) (*8*).

Just as the Human Genome Project did, the desire to eradicate cancer will continue to inspire genomic technology development. The cost of DNA sequencing dropped from more than $10 per nucleotide base in 1990 to less than a penny per base in 2007, and it is expected to drop further with the emergence of innovative sequencing methods (*9*). Because of these and other technological developments, large-scale approaches that were unthinkable even a few years ago have emerged as perhaps the most efficient and cost-effective way to identify the wide array of genomic factors involved in cancer. There seems little doubt that progress in this field will continue to improve our view of this disease.

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10.1126/science.1151428

PLANETARY SCIENCE

Hidden Mars

Peter H. Schultz

Satellite radar signals suggest that something unusual is buried beneath one of the most enigmatic regions of Mars.

Thick sedimentary deposits cover more than one million square kilometers in some equatorial regions of Mars. Today, winds extensively erode and redeposit these materials. On page 1125 of this issue, Watters *et al.* (*1*) provide an intriguing look inside one of these deposits—the Medusa Fossae Formation (MFF)—and conclude that they must be a low-density material or that they contain ice. This conclusion is based on radar signals from the Mars Advanced Radar for Subsurface and Ionospheric Sounding (MARSIS) instrument on the Mars Express mission launched by the European Space Agency. Both scenarios have substantial implications for the origin of this vast deposit and similar deposits elsewhere.

Early studies based on Mariner 9 (*2*) and Viking images (*3*) demonstrated that the MFF must be composed of easily eroded materials, as inferred from the extensive wind-eroded landforms and impact craters in various states of destruction. Because the materials drape different topographies, elevations, and geologic units, researchers have interpreted them as air-fall deposits (*4*). The nature and source of these deposits, however, have been debated over the past 30 years.

The MFF deposits were initially thought to be ash from catastrophic eruptions similar to those on Earth that produce vast hot ash flow deposits (*5*, *6*), in contrast to the nonexplosive Hawaiian-like basaltic volcanoes presently visible on Mars (e.g., the volcanic mountain called Olympus Mons). If so, the vents for the proposed violently erupted material must now be hidden beneath the ash deposits (*6*) or these vents formed during the earliest eruptions in the volcanic region called Tharsis but have now been covered over by later nonexplosive lava flows (*7*).

Alternatively, the MFF might be ancient ice-rich deposits resembling the thick, layered terrains at both poles (*4*). This hypothesis was

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based not only on morphologic similarities but also on similar erosional processes typified by terrains near the present poles. For example, distinctive "pedestal craters" occur around the margins of the thick layered terrains at both the poles and the MFF.

Planetary scientists first recognized pedestal craters in the heavily etched plains at high latitudes (>± 50°) and studied them to assess erosion rates (*8*). High-latitude pedestal craters tend to be small (<5 km) and have pedestal heights typically less than 200 m (*9*). Pedestal craters in the MFF, however, are much larger (>10 km in diameter) and more abundant in a given area (see the figure) (*4*). These pedestals are also much taller (as high as 2 km), thereby indicating much greater erosion of the surrounding deposits. In some cases, compressed (or lithified) materials on crater floors result in inverted topography (floor now standing above the surroundings), because the materials around them have been completely removed (*4*).

One mechanism that can produce the pedestal craters is armouring the underlying deposits by a fine layer of ejecta (*8*). Their high-latitude concentrations, however, suggest that they must have formed in deposits of ice and dust left over from past climate cycles (*4*, *6*). During warm periods, embedded frozen volatiles sublime and remove the "glue" for the deposits. However, a layer that protects the ice-rich deposits, except at the exposed edges, can result from a combination of the deposition of ejecta and impact-generated blast effects, including intense winds and heating (*10*).

The similarity in selective erosion around impact craters both at high latitudes and around the MFF (as well as Arabia) does not explain how the inferred ice-rich deposits accumulated at the equator. Three mechanisms have been proposed: ancient polar deposits left behind after polar wandering (*4*), accumulations of equatorial ices during periods of extreme obliquity changes (*6*, *11*, *12*), and ash and volatiles from nearby volcanic eruptions (*13*).

Polar wandering was proposed to explain the enigmatic erosion of these deposits (*4*). The age of the deposits, however, was not determined by the number density of craters on top of the MFF but by the number and size of pedestal craters around the periphery. This was necessary because craters within the thicker portions of the deposits can be reburied or removed, resulting in an underestimate of the true age (just as at the present polar layered terrains). Pedestal craters around the periphery of the deposits, however, can be used to establish a minimum age. A specific prediction was the presence of ancient polar ices trapped underneath.

Ancient polar wandering is supported by the distribution of grazing impacts, possibly caused by an ancient family of equatorial satellites (*14*). And remanent magnetic fields indicate that the magnetic pole was once in the general region of the MFF (*15*–*18*), although the precise polar wander path may be difficult to pin down without more data. The other two mechanisms also can trap ice and dust near the equator. However, they have greater difficulty accounting for the occurrence of these deposits in specific regions on opposite hemispheres, rather than forming an equatorial band.

Watters *et al.* provide evidence that ices may yet be hidden near the martian equator. These results cannot yet prove (or disprove) what process produced the deposits. They do, however, demonstrate that penetrating radar can be used to explore a hidden Mars. If the MFF deposits resulted from volcanic eruptions, then they provide a clue to a very different eruptive style early in martian history and may contain volatiles released from the deep interior. If the MFF deposits resulted from periods of extreme obliquity, then the embedded ices contain a record of climates over the past 3 billion years, and if these deposits are relicts of an ancient pole location, then they not only may hold clues about the atmosphere and mobile materials from 3.5 to 4 billion years ago but also may preserve evidence of past life.

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Old impacts. Relict craters around the edge of the Medusa Fossae Formation on Mars. The crater on top of the ragged, circular plateau is a pedestal crater. Numerous other smaller crater relicts are nearby. The nearly complete removal of the original deposits in which such craters form suggest that the deposits are ice-rich, easily removed during a change in climate. One interpretation of the new MARSIS data indicates that ice may still be buried within these relict equatorial deposits.

INTRODUCTION

A Robotic Future

IN DOUGLAS ADAMS' SCIENCE FICTION SERIES *THE HITCHHIKER'S GUIDE to the Galaxy*, the Sirius Cybernetics Corporation defined a robot as "your plastic pal who's fun to be with," but it was a corporation whose complaints department occupied the major landmasses of three planets and was the only division to constantly show a profit.

Technology and automation have provided a huge number of labor-saving devices and have at times relieved humans from having to do monotonous and dangerous jobs such as painting sheet metal or cleaning oil pipelines. However, there are times when one really needs to interact with another human being, and at these times, the automated "customer service" or banking machine is a poor substitute for a human.

What differentiates robots from other pieces of technology is their ability to combine automation with action and at times a considerable amount of mobility. They are not only able to do static tasks such as dispense money or direct phone calls, but they can also lift, walk, weld, paint, vacuum, or explore. Unlike most devices that are designed to be used by a human operator, robots are increasingly being built to function within a human environment and are thus becoming more and more humanoid. Perhaps for this reason we view them in a different light than other technologies.

This special section looks at robots and robotics from a wide range of perspectives. Bellingham and Rajan (p. 1098) tell how robots with an increasing sense of autonomy are being used to explore the hostile environments under the oceans and in outer space. Pfeifer, Lungarella, and Iida (p. 1088) examine recent efforts to design robots based on lessons learned from biological organisms. They show that robots can improve their performance by borrowing living body plans and substructures. Madden (p. 1094) reviews the progress that has been made in developing artificial muscles that can compete with the properties of human muscle and may one day enable untethered robots to run, leap, jump, or climb. But even as robots become more lifelike, the biological function of self-replication still eludes them, as Cho (p. 1084) reports.

The last two pieces take us from body to brain. In a story by Lester (p. 1086), we find that robots are increasingly used in secondary schools and undergraduate programs as tools to interest students in engineering and computer science. From a different direction, a Perspective from Edelman (p. 1102) describes a research program in which robots equipped with brainlike devices learn to carry out tasks in the presence of visual cues and other sensory feedback. These "Darwin" bots may teach us something about our own ways of thinking and learning.

Outside the special section are two stories in News Focus. Service (p. 1056) looks at future exploration of the Northeast Pacific Ocean using partly robotic platforms, and Cho (p. 1060) covers the DARPA Urban Challenge, an international competition for self-navigating driverless cars held in California earlier this month. In looking to the future it is also prudent to look at the past. In an Editorial by Sawyer (p. 1037), we learn how the science fiction literature has long considered a robotic future and the many ethical questions this will raise.

They most certainly won't be all plastic, and they may not be our pals, but robots are going to play an increasing role in both scientific and everyday life. It will certainly be interesting to see where we take them and where they are able to take us.

–MARC S. LAVINE, DAVID VOSS, ROBERT COONTZ

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Robotics

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Science

NEWS

Making Machines That Make Others of Their Kind

For decades, self-replicating robots have been a roboticist's dream—and a science-fiction writer's nightmare. Yet engineers haven't found a way to create 'bots that beget 'bots

As any sci-fi fan knows, monkeying with robots ultimately leads to mass carnage. From *R.U.R. (Rossum's Universal Robots)*, the play that in 1921 introduced the word "robot," to the battles with the Daleks in the television show *Doctor Who*, to the *Terminator* movies, the tale has been told time and again. Humans (or humanoid aliens) foolishly make robots that reproduce. The self-replicating robots decide that people are a nuisance and set out to exterminate them. This scenario might seem less farfetched now that robots can make cars and microchips and stalk terrorists from the skies.

Don't panic just yet. Vicious self-replicating machines resembling Arnold Schwarzenegger won't be breaking down doors anytime soon. Anyone mighty enough to kick a toy or topple blocks can overpower today's self-replicating robots, which actually need a lot of help to make something identical to themselves. Self-replication "is fundamental to nature and at the core of evolution, and yet we have no idea how to do it with synthetic systems," says engineer Hod Lipson of Cornell University. "That's always been a sore point for robotics."

A handful of researchers are striving to change that. Working on shoestring budgets and with materials associated more often with child's play than research, they've developed simple robots that can make others like themselves out of a few relatively complex parts. They're defining more precisely what it means for a machine to self-replicate. And some are striving to emulate nature's knack for reproduction. Progress has been modest—stacks of blocks that stack other blocks won't conquer the world—but researchers are optimistic that, at the very least, they may soon better understand exactly what problem they're trying to solve.

All agree that progress has been slowed by a lack of funding, as self-replicating robots serve no earthly purpose—although in theory, they could be useful in establishing a base on the moon or on Mars. "The field is, like, three people," says mechanical engineer Gregory Chirikjian of Johns Hopkins University in Baltimore, Maryland. Researchers face conceptual barriers as well. "There is a great need to come up with the basic scientific principles" of self-replication, says aerospace engineer Pierre Kabamba of the University of Michigan, Ann Arbor. Still, researchers have taken intriguing steps toward making machines that build copies of themselves.

Easy, in theory

The notion of self-replicating machines stretches back centuries. But the rigorous theory of self-replication emerged in the 1940s and 1950s, when mathematician John von Neumann, who also laid much of the groundwork for modern computing, analyzed the problem.

Von Neumann considered a collection of automata: self-guided cell-like entities that interact according to specific rules. He wondered what tasks a clump of them would have to do to replicate from raw materials and basic parts. The thing would have to consist of at least three subunits, he figured: first, a set of instructions for making a device; then, a unit that reads those instructions to make a new device; and finally, one that copies the instructions, which von Neumann envisioned as a coded tape.

This agglomeration would read the tape, make its progeny, and pass a copy of the tape to its offspring. The scheme bears a striking resemblance to biology, in which cells replicate by reading and copying tapelike molecules of DNA, the structure of which was discovered after von Neumann cooked up his ideas. Spurred by von Neumann's work, computer scientists and others have designed myriad programs that replicate within a computer—including viruses and worms.

But as a plan for making self-replicating machines, von Neumann's work left much to be desired. Like a true mathematician, he skipped over the practical difficulties a real machine would have in gathering parts. "He doesn't address the physics at all," Lipson says. "Bringing in the materials, dealing with the errors—the physics is the difficult part."

Give a child a Lego set, and she will immediately dump the pieces on the floor and comb through them to find the ones she wants. That's precisely the task that stumps machines. "That's not just the hard part for self-replication, it's the hard part for robotics in general," Chirikjian says. "The reason you don't have robots doing your dishes and walking your dog is that the world is very complicated, and it's difficult for a robot to handle it."

Picking up the pieces

So some engineers give their robots a helping hand. Two years ago,

On track. Engineer Gregory Chirikjian's robots must follow a specific path to replicate.

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Lipson and colleagues unveiled programmable blocks measuring 10 centimeters across. Each consisted of two pyramid-shaped halves that could swivel against each other, and each block could grip others using magnets on its faces. Wriggling like a drunken hula dancer, a stack of four blocks could assemble a second stack, if new blocks were fed in at the right place and times, the researchers reported in the 12 May 2005 issue of *Nature*.

Although one stack of blocks does form another, it still seems a far cry from a fully self-replicating robot. Instead of some basic part, each cube is itself a fairly sophisticated robot. And the contorting tower requires plenty of human assistance to help it locate the additional blocks. To produce something truer to the spirit of self-replication, Lipson is now experimenting with simpler cubes measuring only 500 micrometers wide that jumble together randomly in a fluid. "What is the smallest building block from which we can make everything?" Lipson says. "That's the crucial question."

Chirikjian also began with robots that assembled others from a few complex chunks. Starting in 2002, he and his students began experimenting with robots made of Lego bricks. At first, they built remote-controlled vehicles that could be broken into a few components. When placed in a pen, one robot could push the components of another together—a crude form of self-replication, given that the guts of a robot lay mostly in the one component containing the computerized controller.

Since then, Chirikjian and his students have striven to make their robots more autonomous and to assemble them from simpler parts. They developed a system of optical sensors that allows a robot to follow a colored stripe to find various parts. They have simplified the robots by replacing the central controller with cruder electronics distributed throughout the pieces. Recently, the researchers demonstrated a self-replicating robot made of six fairly simple modules, and Chirikjian and a grad student are working on one consisting of 100 pieces.

Chirikjian's robots look more or less self-sufficient, but they do not truly forage for parts. Rather, they depend on a track to guide them. Chirikjian says that he is working to eliminate the track. But he notes that even biological systems depend on their environment to reproduce. "If you take the DNA out of the environment of the cell, it's no longer self-replicating," he says.

Doing what comes naturally

Given the challenges of step-by-step, or deterministic, assembly, some researchers are opting for chaos instead. Rather than making their robots fetch pieces, they're relying on random collisions to bring parts to the robots in efforts that mimic the mingling of biomolecules in cells.

CREDIT: CORNELL UNIVERSITY

Basic parts? A stack of Hod Lipson's cubes stacks more cubes, but each is itself a complex robot.

For example, as a graduate student at the Massachusetts Institute of Technology (MIT) in Cambridge, materials scientist Saul Griffith developed smart tiles that can latch onto one another as they glide and jumble on an air table. Whether two tiles latch depends on how they are already connected to other tiles. When the tiles were properly programmed, a chain of them could form another chain, Griffith and colleagues reported in the 29 September 2005 issue of *Nature*. "In many respects, self-replication is just a party trick," says Griffith, now president of Makani Power in Alameda, California. "You don't even need much logic."

The random, or "stochastic," approach may have a key advantage. Ironically, jumbling huge numbers of pieces together should be easier than putting them together one by one, says engineer Eric Klavins of the University of Washington, Seattle, who has developed a similar set of triangular tiles. "If you want to do self-replication with billions of parts, you're not going to get away with determinism," he says. The stochastic approach presents its own challenges, however. For example, researchers must figure out how to form larger useful structures from the pieces while preventing them from glomming together in undesirable ways.

A few molecular biologists are even pushing to develop artificial cells. For such research, the emphasis is a little different, says Jack Szostak of Harvard Medical School and Massachusetts General Hospital in Boston. In chemistry, self-replication is fairly common, as any chemical that catalyzes its own production does it. Szostak and colleagues are striving for something more. "What we're trying to do is to develop a self-replicating chemical system that can evolve," Szostak says.

For the membranes for his artificial cells, Szostak employs molecules called lipids, which can form fluid-filled shells. Within the shells, he hopes to store a length of DNA, RNA, or a related molecule that can store coded information, replicate, and mutate. The researchers have already shown that they can make the shells grow and divide—by forcing them through a small pore—and they are working on the material to store within the shells.

Researchers have a long way to go, however. For example, molecular biologists have been searching for a strand of RNA called a ribozyme that can catalyze the replication of itself and other strands. Such a ribozyme would have to churn out strands a couple of hundred chemical letters long, but so far the best candidate can string together only about 20 letters. "Twenty years ago, I thought this would be a 20-year project," Szostak says. "Maybe it still is."

Waiting for the Terminator

Where research on self-replication will lead remains unclear. Some say that practical considerations will inevitably force researchers toward biomolecular systems. "Self-replicating robots are going to be made out of biomolecules long before bulldozers start copying themselves," Griffith says. Others say it's not so clear that self-replication in synthetic biology is easier than in mechanical robotics. "You're comparing two very difficult things," says molecular biologist David Bartel of MIT. "So which one is more difficult may not matter."

Meanwhile, some say that the concept of self-replication needs a rethink. Researchers have thought that a system is either self-replicating or it isn't, Lipson says. But given that even biological systems rely heavily on their environment, it seems there are different shades of self-replication. Both Lipson and Chirikjian have developed mathematical tools to quantify them. Using them, researchers might analyze a system to figure out how to make it more self-replicating, Lipson says.

Of course, employing such scales, one might argue that self-replicating robots already exist. Machines are typically made by other machines these days, albeit with plenty of help and guidance from humans. So perhaps the entire industrial enterprise constitutes a swarm of self-replicating robots. That seems plausible. But it also seems to be a disappointingly long way from the grand vision of machines that don't need people. Maybe that's a good thing.

–ADRIAN CHO

NEWS

Robots' Allure: Can It Remedy What Ails Computer Science?

Faced with sagging enrollments in the field, school and university instructors are engineering a deus ex machina to turn things around

When Elizabeth Sklar's class starts, the first thing her students do is reach for their Lego bricks. This isn't kindergarten; it's an introductory computer science course at Brooklyn College, one of 19 campuses of the City University of New York, and the Lego bricks are a far cry from the ones you built houses and towns from as a child. They're Mindstorms RCX, Lego's programmable robotics kit, and for Sklar, a computer scientist at the college, they are bait to get students hooked on computer science. In some ways, she says, it works too well: "When they get the robots in their hands, they don't want to do lectures, I can't get them to leave, and they want to take the robots home with them."

Her first assignment has the students, most of whom have never programmed before, code their wheeled robots to drive forward in a straight line for 6 seconds. By the end of the first lab, some are tracing spirals, and by the end of the robotics unit, a month and a half later, the robots are using sensors to see and feel their environment.

The same things are happening in college and secondary school classrooms across the United States and in the Middle East, Europe, Asia, and South America. Everywhere the goal is identical: to attract more students to computer science.

Over the past 30 years, undergraduate computer science enrollments at universities in the United States have followed a roller-coaster–like trajectory. After peaking during the PC revolution of the early 1980s and the dot-com boom of the late 1990s, the number of students pursuing a computer science major has fallen significantly. At Stanford University in Palo Alto, California, for example, the number of undergrads declaring a computer science major dropped from 171 in 2000–2001 to 86 in 2006–2007. Women, always a small minority in the field, have become even scarcer than before. The Higher Education Research Institute at the University of California, Los Angeles, reports that in 2004 fewer than 0.5% of female college freshmen were interested in computer science as a major—a low not seen since the early 1970s.

Experts trace the drop in numbers to students' concerns about job prospects in the wake of the bursting of the dot-com bubble, increased offshoring, and the influx of cheap skilled labor from abroad. Many, however, think students are unduly pessimistic about their opportunities. And some, including Owen Astrachan, a computer scientist at Duke University in North Carolina, say the lack of interest has another key component: The classes are boring.

'Bots got game. In Botball tournaments, automata from around the world compete in miniature arenas.

"The classic way to teach computer science is to [give] a really dry assignment like 'Write a program to print the Fibonacci sequence,' " says Tucker Balch, a computer scientist at the Georgia Institute of Technology (Georgia Tech) in Atlanta. "Students don't get turned on by this." In response, both Brooklyn College and Georgia Tech have spiced up their computer science classes with gaming, media manipulation, applications to other disciplines such as biology and economics—and robots.

"We're using the sexiness of robots to lure students into computer science," says Douglas Blank, who teaches introductory computer science at Bryn Mawr College, an all-women's college outside Philadelphia, Pennsylvania. Blank, Balch, and their colleagues at Bryn Mawr and Georgia Tech used a $1 million grant from Microsoft as seed money to set up the Institute for Personal Robotics in Education (IPRE) in 2006. Now, when the 180 students in Georgia Tech's introductory computer science course go to the bookstore at the beginning of the semester, each shells out $68.75 for a shrink-wrapped, lunch box–sized blue robot called the Scribbler.

On the first day of class, students tackle their robots' Python programming language, naming the 'bots and driving them in circles. By mid-term at Bryn Mawr, robots named Henry Johnson, Borg, Igor, Mintyfresh, Myro-0001, TevBot, and RJ Nivram, among others, have progressed far beyond circles. Mr. Johnson (operated by freshman Danielle Pan, who has decorated him with stalked eyes and red-felt crab claws) and company are on a mission to seek out an orange pyramid placed in the middle of the Bryn Mawr computer lab, using the robots' cameras. Mission accomplished, the students' computer speakers erupt with customized sound files: the "Hallelujah Chorus," lines from the classic comedy movie *Airplane!*, and simple, loud gloating.

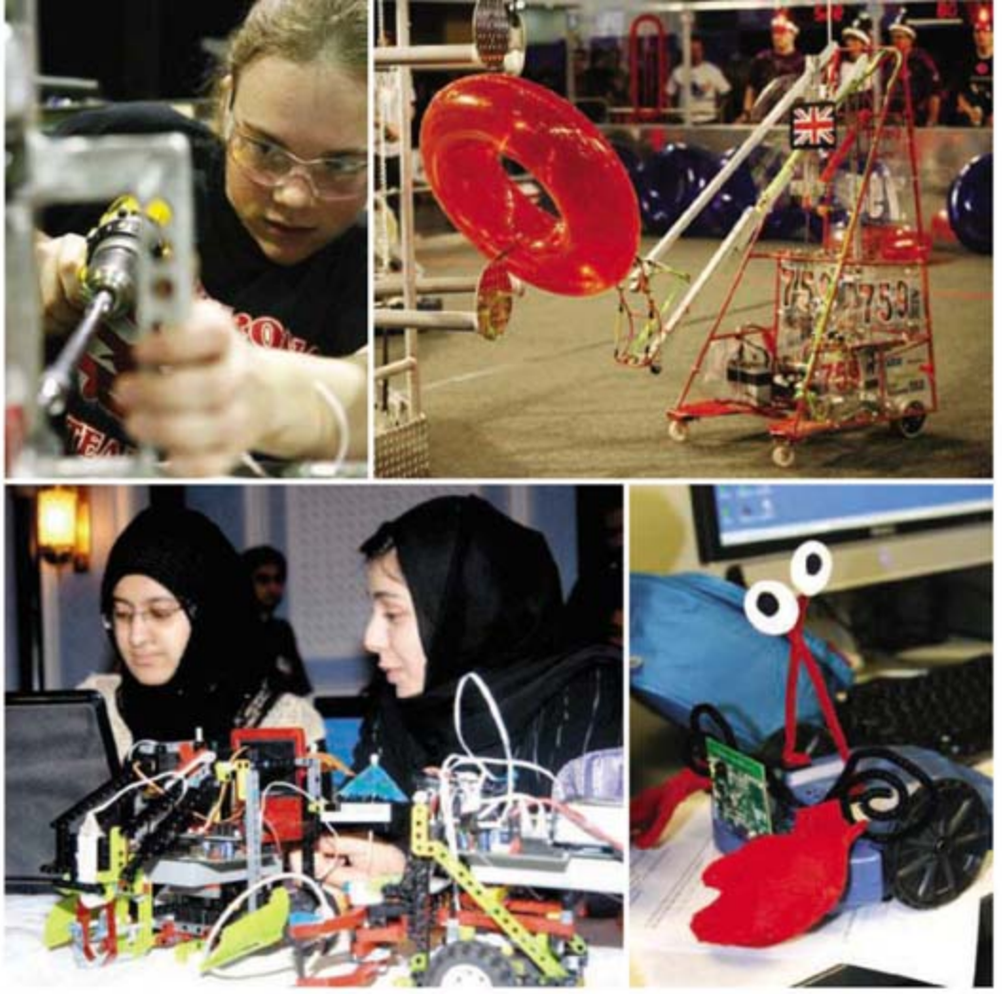
"I came here to be a German major, but now I'm really into computer science—definitely going to be a major," says freshman Stephanie Viggiano. Freshman Rebecca Rebhuhn-Glanz had been planning a math major, but "now I'm thinking computer science—it's too much fun," she says.

Filling the pipeline

Elsewhere, robots are enthralling even younger students. STEM (science, technology, engineering, and mathematics) advocates have been using them for years to reach out to students aged 6 to 18, often through leagues as competitive as any high school varsity sport.

Botball is one such activity, run by the KISS (Keep It Simple, Stupid) Institute for Practical Robotics (KIPR), headquartered in Norman, Oklahoma. The 2007 competition pitted 301 teams of secondary school students from the United States and elsewhere in 14 regional Botball tournaments, followed by a championship at the National (now Global) Conference on Educational Robotics in Honolulu, Hawaii. In 90-second rounds,

CREDIT: BOTBALL EDUCATIONAL ROBOTICS PROGRAM



Scenes from all over. (*Top, left to right*) Assembling a robot at the U.S. FIRST high school competition; a British entry takes on "Rack and Roll." (*Bottom, left to right*) Botballers from Qatar; a crab-'bot at Bryn Mawr College.

CREDITS (TOP TO BOTTOM): ADRIANA M. GROISMAN; CS CARNEGIE MELLON, QATAR; B. LESTER/SCIENCE

contestants' machines scurried around a 1.2-by-2.4-meter playing field, vying to save a "village" (sections of PVC pipe, felt balls, and drink umbrellas) from a "volcano" (a tower spewing bright orange plush balls) by moving and sorting objects using a variety of scoops and pincers. Each team had 7 weeks last winter to design, build, and test two fully autonomous robots using Lego bricks, gears, motors, and sensors—all controlled by a KIPR robot controller called the XBC whose brain is a Game Boy Advance. The game stresses innovative design and programming in the XBC's Interactive C language.

The competitors included several teams from the Middle East. Botball spread to the region after the rulers of Qatar invited U.S. universities to set up branch campuses on a new 1000-hectare complex called Education City (*Science*, 5 December 2003, p. 1652). One taker was Carnegie Mellon University (CMU) in Pittsburgh, Pennsylvania. Charles Thorpe, a CMU computer scientist, moved out to become dean and took along his son, Leland, a former Botball player taking a year off before beginning college. With assistance from the university, the Thorpes set up a Qatari Botball league. Interest exploded—from four teams in 2004 to 18 at the 2007 competition, including three each from the United Arab Emirates and Kuwait, says Mohamed Mustafa, who took over as Botball's Middle East coordinator when Leland Thorpe returned to the United States.

The 2007 competition included four all-female teams, one of which, a group of middle-school students from the Al Maha English School for Girls in Doha, placed fourth. Mustafa estimates that the 2008 tournament will see 36 teams, including eight from Bahrain and Egypt. As hoped, he says, some Botballers from years past have ended up at the CMU Qatar campus.

The Hawaii regional, held at the end of April, was won this year by a team of 10- to 15-year-olds from Earl's Garage in Kamuela on the Big Island. Whereas most Botball teams are organized by schools, Earl's Garage is a community club specifically geared to promote STEM. "There's this perception that science is hard or uninteresting," says Earl's Garage founder and Wizard of Wonder Michelle Medeiros. "Robots are a great way to remove some of that fear." After 4 years of Botball competitions, Medeiros is organizing a team to enter a more grueling contest: the FIRST Robotics Competition (FRC).

If Botball is the robotics equivalent of a fencing match, then FRC is more like a really foul-tempered game of ice hockey. The brainchild of Dean Kamen, the inventor of the Segway, FRC is high-energy and heavily sponsored. Whereas Botball robots are small and the focus is on programming, FRC uses 70-kilogram metal robots and concentrates on engineering. After a short "autonomous" period at the beginning of each round, the 'bots are remote-controlled.

Kamen started FIRST (For Inspiration and Recognition of Science and Technology) in 1992, with 28 teams at a single event in New Hampshire. In 2007, 1307 teams comprising 32,500 high school students from Brazil, Canada, Israel, Mexico, the Netherlands, the United Kingdom, and the United States competed in "Rack and Roll"—culminating in world championships at the Georgia Dome in Atlanta. The game tasked two teams of robots with hanging red or blue pool inner tubes on pegs on a large circular tower in a kind of three-dimensional ticktacktoe while trying to fend off 'bots from the opposing team. FIRST calls its high school competition "a varsity sport of the mind." It has spawned two competitions for younger students.

Both Botball and FIRST are expensive to play. Botball materials and registration costs each team $2300, and an FRC team costs between $6000 and $8000. Both organizations have opportunities for financial aid to encourage participation from poorer schools, funded by corporations and governments concerned about STEM shortages.

Besides Microsoft's $1 million grant to IPRE, the U.S. National Science Foundation (NSF) recently announced $6 million in grants to revamp computing education at 25 schools nationwide. FIRST counts GM, Motorola, Xerox, and the Central Intelligence Agency among its 2000 sponsors, and half of all Botball teams receive financial aid through KIPR sponsors that include NASA and the Naval Research Laboratory.

Without a doubt, Botball, FIRST, and a slew of other competitions from around the world—including the World Robot Olympiad, the International Robot Olympiad, and the India Robot Olympiad—have succeeded in funneling students into the STEM pipeline. A Brandeis University study found that FIRST participants were twice as likely to pick college science or engineering majors as were non-FIRST participants who took similar high school math and science courses.

"We need to inspire the best and the brightest to go into computing," says Jeannette Wing, assistant director of NSF's Computer and Information Science and Engineering Directorate. And for better or worse, the way to students' hearts seems to be through their robots.

–BENJAMIN LESTER

REVIEW

Self-Organization, Embodiment, and Biologically Inspired Robotics

Rolf Pfeifer,[1]* Max Lungarella,[1] Fumiya Iida[1,2]

Robotics researchers increasingly agree that ideas from biology and self-organization can strongly benefit the design of autonomous robots. Biological organisms have evolved to perform and survive in a world characterized by rapid changes, high uncertainty, indefinite richness, and limited availability of information. Industrial robots, in contrast, operate in highly controlled environments with no or very little uncertainty. Although many challenges remain, concepts from biologically inspired (bio-inspired) robotics will eventually enable researchers to engineer machines for the real world that possess at least some of the desirable properties of biological organisms, such as adaptivity, robustness, versatility, and agility.

Although traditionally, biologically inspired (bio-inspired) robotics has been largely about neural modeling (for example, for phonotaxis, navigation, or vision), recent developments in the field have centered on the notions of self-organization and embodiment; that is, the reciprocal and dynamical coupling among brain (control), body, and environment. We will show that most advances converge onto a set of principles that are implicitly or explicitly employed by robot designers: First, the behavior of any system is not merely the outcome of an internal control structure (such as the central nervous system). A system's behavior is also affected by the ecological niche in which the system is physically embedded, by its morphology (the shape of its body and limbs, as well as the type and placement of sensors and effectors), and by the material properties of the elements composing the morphology (*1*). Second, physical constraints shape the dynamics of the interaction of the embodied system with its environment (for example, because of the way it is attached to the body at the hip joint, during walking a leg behaves to some extent like a pendulum) and can be exploited to achieve stability, maneuverability, and energy efficiency (*2*, *3*). Third, a direct link exists between embodiment and information: Coupled sensory-motor activity and body morphology induce statistical regularities in sensory input and within the control architecture and therefore enhance internal information processing (*4*). Fourth, viewing an embodied agent (*5*) as a complex dynamical system enables us to employ concepts such as self-organization and emergence rather than hierarchical top-down control. As we review some of the recent advances in bio-inspired robotics, it will become clear that autonomous agents display self-organization and emergence at multiple levels: at the level of induction of sensory stimulation, movement generation, exploitation of morphological and material properties, and interaction between individual modules and entire agents.

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Bio-Inspired Embodied Systems

Artifacts in general and robots in particular are always designed for a particular task environment in which they have to achieve certain behaviors. In a manufacturing plant, where robots have to rapidly weld pieces together, precisely assemble motors, or neatly package chocolates into boxes, the focus is on speed, precision, controllability, and cost-effectiveness. In contrast, robots having to perform in the real world should be able to cope with uncertain situations and react quickly to changes in the environment. Biological systems provide an exceptional source of inspiration. The biological world is immensely diverse—roughly 1.5 million different species have so far been identified—and this richness is also, though at a much smaller scale, reflected in the different types of robots that have been developed (table S1). Bio-inspiration originates from molecular and cellular reproduction (*6*–*8*); slime molds (*9*); walking insects (*10*, *11*); flying insects (*12*, *13*); spiders (*14*); lobsters (*15*); octopuses (*16*); fish (*17*) and other aquatic creatures; amphibious animals such as salamanders (*18*) and snakes (*19*); four-legged animals such as geckos (*20*), mice (*21*), and dogs (*17*, *22*); and, of course, primates such as monkeys and human beings (*23*–*25*). Major goals for these robots are movement, locomotion (crawling, walking, running, climbing, swimming, and flying), navigation, orientation, manipulation, imitation, and cooperation. Biology contains especially rich and useful knowledge for robotics in disciplines such as neuroscience (in particular, computational neuroscience and neuroethology), biomechanics, animal physiology, and systems biology.

Given the vastness of the information available, the question arises as to what insights from biology could and should be exploited for designing robots. Simply copying a biological system is either not feasible (even a single neuron is too complicated to be synthesized artificially in every detail) or is of little interest (animals have to satisfy multiple constraints that do not apply to robots, such as keeping their metabolism running and getting rid of parasites), or the technological solution is superior to the one found in nature (for example, the biological equivalent of the wheel has yet to be discovered). Rather, the goal is to work out principles of biological systems and transfer those to robot design. This philosophy underlies, for instance, the rapidly expanding field of bionics, which seeks to design technology by mimicking the salient features of biological structures (*26*). One important lesson from bionics studies is that successful natural designs rely on effective embodiment: on clever morphology and use of material properties.

If properly applied, embodiment can lead to surprising insights. Although the idea has been around for quite some time (*27*–*29*), its implications for the design of autonomous adaptive systems have not yet been sufficiently explored and theoretically elaborated. As a consequence, robot designers often opt for centralized solutions where there is a microprocessor responsible for controlling the movement of all limbs and joints. Simply applying methods from control engineering to robots that have to perform in the real world has not worked well in practice: many humanoid robots, for example, are still energetically inefficient and lack adaptivity when confronted with situations that animals cope with on a routine basis. An embodied perspective, because it distributes control and processing to all aspects of the agent (its central nervous system, the material properties of its musculoskeletal system, the sensor morphology, and the interaction with the environment), provides an alternative avenue for tackling the challenges faced by robotics. The tasks performed by the controller in the classical approach are now partially taken over by morphology and materials in a process of self-organization; for example, skin properties support the functionality of hands: Grasping a glass with soft, compliant, slightly humid fingertips is much easier than with thimbles, because the deformation of the tissue on the fingertips, which is entirely passive, increases surface contact and friction. Clearly, the embodied view suggests that the actual behavior emerges from the interaction dynamics of agent and environment through a continuous and dynamic interplay of physical and information processes (Fig. 1). Although important insights can be gained from simulations, most of this review is devoted to studies employing physically embodied robots; indeed, in spite of recent advances in simulation technology, the actual dynamics of the real world are still very hard to simulate accurately (such as the interaction of an agent's body with sand or water).

Embedded Neural Models

Historical precursors of today's bio-inspired robots were the mechanical tortoises built by Grey

Walter in the 1940s; these machines displayed an impressive behavioral repertoire based solely on neurally inspired analog electronics. In the past 15 years or so, robots of all sorts have been used to study and test models of natural neural information processing [for recent surveys, see (*1*, *30*, *31*)]. Bio-inspired neural modeling has driven research on locomotion; for instance, to understand legged underwater locomotion (*15*), to study the switching between swimming and walking observed in salamanders (*18*) (Fig. 2A), or to investigate adaptive dynamic walking on irregular terrain (*22*). Much attention has also been devoted to emulating navigation and orientation behavior. Examples abound and include visual homing inspired by how bees or wasps find their way back to their nests (*12*), cricket phonotaxis [how female crickets move toward the mating sounds of males in highly rugged and noisy environments (*32*)], and spatial memory formation by modeling place fields and head-direction cells which account for the sophisticated navigational skills of rodents (*33*). One of the important design principles implicitly exploited in the examples above is sensory-motor coordination (*34*); that is, the mutual coupling of sensing and acting. This principle supports the generation of information structure in sensory stimulation: spatiotemporal correlations in sensory input streams, redundancies between different perceptual modalities, or regularities in sensory patterns that are invariant with respect to changes in illumination, size, or orientation.

The information-theoretic implications of embodiment are far-reaching. First, the induced information structure represents redundancy across sensory channels, which may, given the typically staggering number of possible states that the sensory input can assume, substantially simplify perception. Second, information structure does not exist before the interaction occurs but emerges only when the embodied system interacts with its surroundings. However, once such structure has been induced, learning can pick up on it by forming cross-modal associations, so that next time around, the pertinent information structure is more easily reactivated, and, for example, stimulation in one sensor modality can be partially predicted from another one (for instance, by looking at a glass we can partially predict what it will feel like when we grasp it). It follows that embodied interaction lies at the root of learning because it enables the creation of time-locked correlations and the discovery of regularities that transcend the individual sensory modalities (*4*) as necessary for concept learning, such as the grasping of a cup, which yields visual, haptic, and proprioceptive sensory information.

Implications of Embodiment

In the examples discussed so far, the importance of using robots lies mostly in the fact that the neural models are embedded in an embodied system equipped with sensors and actuators enabling physical interaction with the environment. The models are thus exposed to realistic sensory stimulation, rather than the idealized one typically used in simulation studies (Fig. 2B). Recently, there has been a growing interest in a more integrative study of biologically inspired systems, and many of the implications of embodiment have shifted toward center stage: exploitation of morphology and materials, sensory-motor interaction dynamics, and self-organization (or more precisely, self-stabilization, which is stabilization without explicit feedback mechanisms; that is, without measuring the disturbances or altering the system) (*3*, *10*, *17*, *22*, *35*) (Fig. 3).

As an illustrative example, take legged locomotion in insects. Insects possess dozens of degrees of freedom that need to be coordinated during walking or running, which is particularly challenging when dealing with uneven surfaces. It is plausible to assume that insects do not solve the inverse kinematics problem for all the joints at all times (a strategy often adopted in robotics, but which though being computationally exact requires high-bandwidth sensory feedback for fast gaits). The solution to the control problem can be found in the exploitation of embodiment and decentralization. If the insect is pushing back with one leg, the joints of all the other legs that are on the ground are moved in the "correct" direction, a movement that can be detected by angle sensors in the joints (*11*). This way, there is global communication between the legs that can be exploited for their coordination, even though at the level of the neural system no central controller for the legs exists.

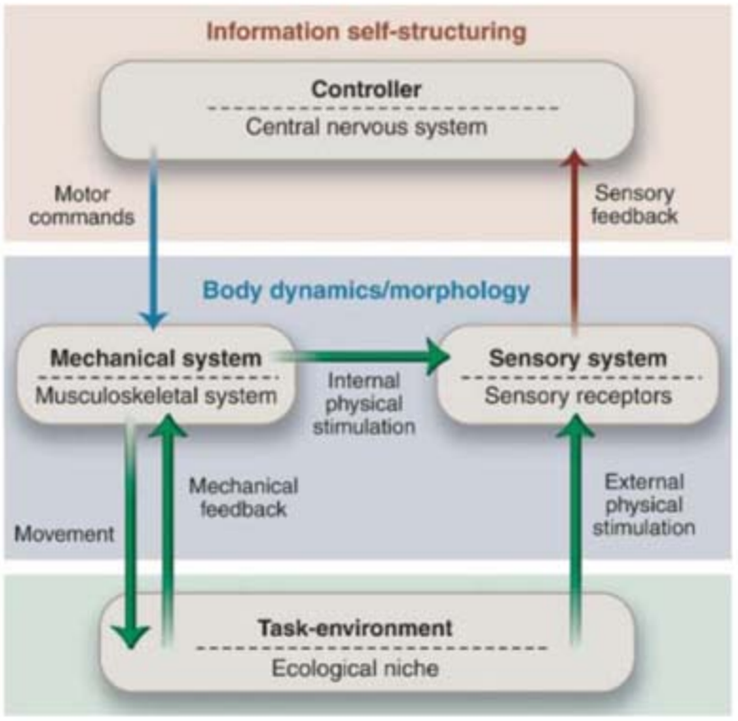

Fig. 1. Implications of embodiment (the interplay of information and physical processes). Driven by motor commands, the musculoskeletal system (mechanical system) of the agent acts on the external environment (task environment or ecological niche). The action leads to rapid mechanical feedback characterized by pressure on the bones, torques in the joints, and passive deformation of skin tissue. In parallel, external stimuli (pressure, temperature, and electromagnetic fields) and internal physical stimuli (forces and torques developed in the muscles and joint-supporting ligaments, as well as accelerations) impinge on the sensory receptors (sensory system). The patterns induced thus depend on the physical characteristics and morphology of the sensory systems and on the motor commands. Especially if the interaction is sensory-motor coordinated, as in foveation, reaching, or grasping movements, information structure is generated. The effect of the motor command strongly depends on the tunable morphological and material properties of the musculoskeletal system, where by tunable we mean that properties such as shape and compliance can be changed dynamically: During the forward swing of the leg in walking, the muscles should be largely passive, whereas when hitting the ground, high stiffness is required, so that the materials can take over some of the adaptive functionality on impact, such as the damped oscillation of the knee joint.

Another means to simplify the control of dynamic locomotion is through passive mechanisms alone. For instance, the rapid adaptation to small unpredictable bumps in the ground is taken over by the passive compliance of the insect's musculotendon system and the slack in its joints (*3*, *14*). Technically, it is possible to realize this principle for hexapod walkers using pneumatic linear actuators (air muscles), where the compliance is provided by compressed air, enabling the robots to move at impressive speeds even over rough terrain (*10*) (Fig. 2C). Similar feats have been achieved by employing electrical motors coupled to spring-damper systems in the case of quadrupeds (*17*, *22*).

These are all examples of what one might call "intelligence by mechanics" (*35*), which implies that the intrinsic dynamics of the nonlinear mechanics yield self-stabilizing behavior (that is, robustness with respect to perturbations with minimum neural sensing). Paradigmatic examples of self-stabilizing systems are the passive dynamic walkers: robots (or rather mechanical structures without microprocessors or motors) that walk down a slope without control and actuation (*2*). The walker's morphology (center of

mass, length of the limbs, and the shape of the feet) and its materials are carefully designed so as to exploit the physical constraints present in its ecological niche (friction, gravity, and inclination of the slope) for locomotion. Interestingly, to get the robot to learn to walk on a level surface, one can reuse the mechanical design obtained during passive dynamic walking and endow it with actuators in the ankles or hips (Fig. 2D). The natural dynamics of the body/environment system can be used as a target for learning the control policy of the actuators, and it is an "easy" one because once the system is in the desired basin of attraction, it is "pulled" into a quasiperiodic limit cycle trajectory. In other words, the robot learns to walk on flat ground within a relatively short period of time. The theoretical import of this case study lies in the tight coupling of embodiment, self-organization, and learning. Again, the ability to walk is not localized in the controller but is fully distributed throughout the agent and its dynamics; part of the control task is "outsourced" to the physical dynamics of the agent.

In all forms of locomotion, neural control can be simplified through clever morphological design and use of functional materials. A case in point is aquatic locomotion. It is known that fish, some amphibians, and reptiles swim by producing traveling waves of neural activation transmitted along chains of coupled oscillators located in the spinal cord (*36*). The attempt to model and mimic the very same mechanism has inspired a host of multisegmented undulatory robots such as fish, swimming salamander, and snake robots (*18*, *19*). The key to their construction is the translation of neural

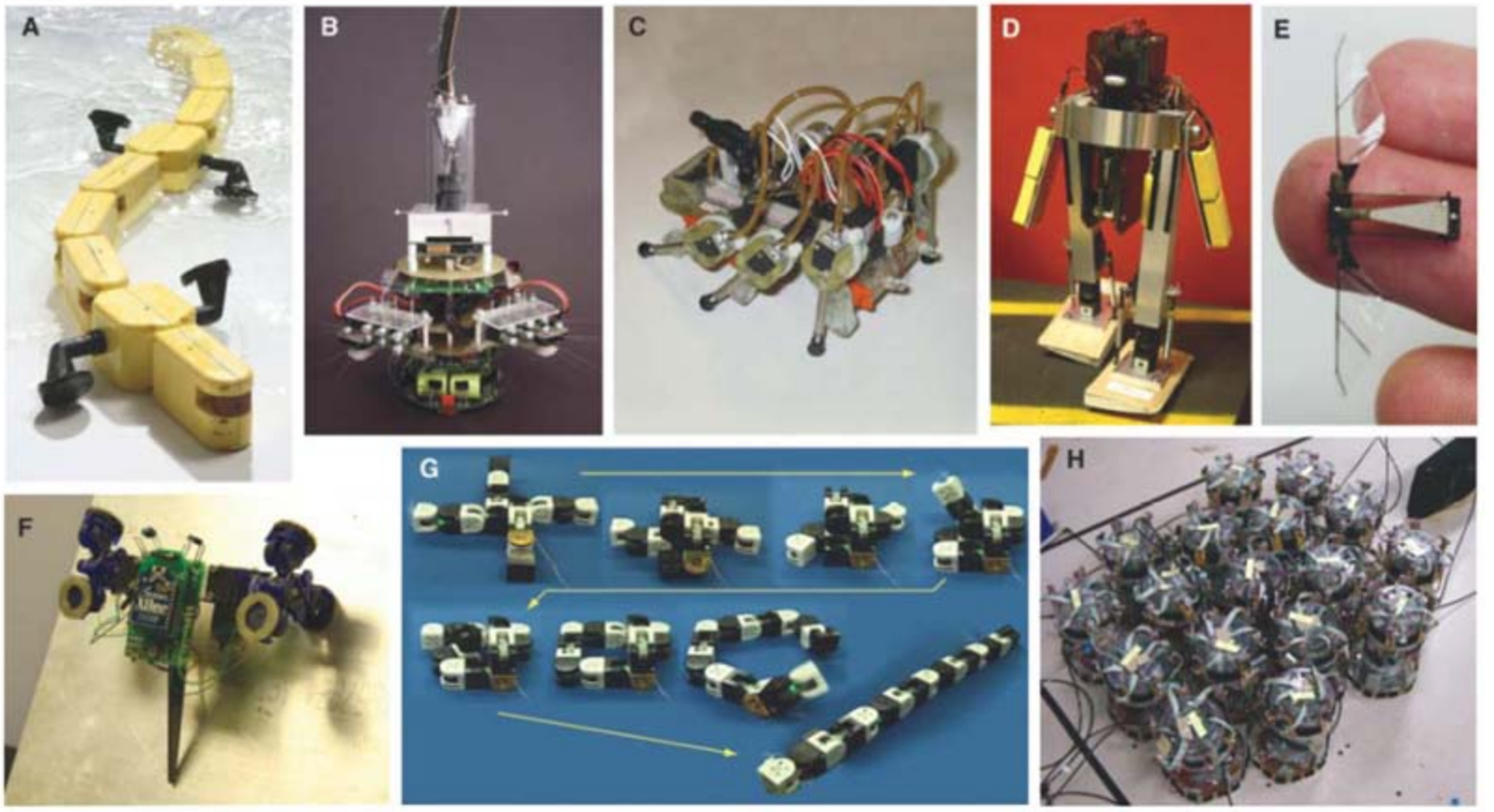

Fig. 2. Self-organization, dynamics, and materials in bio-inspired robotics. (**A**) Smooth transition between swimming and walking (*18*). This amphibious salamanderlike robot (~80 cm long) embeds a spinal cord model that explains the ability of salamanders to switch between swimming and walking. The locomotion model is built by extending a primitive neural circuit for swimming by phylogenetically more recent limb oscillatory centers. (**B**) Rich sensory stimulation through proper sensor morphology (*21*). This robot (~7 cm in diameter) owes its sophisticated sensory capacities to the specific arrangement, shape, and material characteristics of its whiskers. Natural whiskers from rodents (such as the ones used on this robot) are far superior to whiskers built from other materials in terms of richness of the signals relayed to the neural system. (**C**) Self-stabilizing rapid hexapod locomotion (*10*). This robot (~15 cm long) moves with a bouncing gait, achieving rapid (over 4 body lengths per second) locomotion. Its legs are built with compliant pneumatic actuators, which yield self-stabilization through mechanical feedback. (**D**) Passive dynamics–based walking (*2*). Designed to work on a slope as a dynamic walker, this robot (~45 cm tall) exploits dynamics and morphology (in particular, the shape and length of the body and feet) to achieve stable walking. The robot's natural dynamics serves as the target dynamics for a reinforcement learning mechanism, enabling the robot to quickly learn to walk on flat ground. (**E**) Self-stabilizing vertical takeoff through materials and morphology (*13*). Inspired by flies, this ultralight (60 mg, 3-cm wingspan) ornithopter (a device that flies by flapping its wings) generates sufficient lift to take off vertically (power is supplied externally). A large part of the control is delegated to the morphological and material properties of the robot. Compliant structures are driven into resonance to produce a large wing stroke, and flexible material is used in the wing hinges to allow for passive rotations of the wings. (**F**) Agile wall-climbing through materials (*20*). The bio-inspiration for this palm-sized robot is provided by the gecko and its uncanny climbing talents. The robot's tri-foot (three-footed wheel) is equipped with a polymer dry adhesive material, which to some extent has contact properties comparable to those of its biological analog. The robot can flexibly navigate on smooth vertical and even inverted surfaces. (**G**) Morphing through localized self-reconfiguration (*7*). This self-reconfigurable robot is composed of active (actuated, black) and passive (nonactuated, white) cubic modules (~400 g, ~60 to 65 mm side length). The modules connect to each other through hooks, which enables the robot to change its morphology in a large number of ways. The picture shows the metamorphosis from a four-legged (quadruped) structure to a linear (snakelike) structure. (**H**) Global movement through local interaction dynamics (*9*). The individual wheel-like modules (~10 cm in diameter) constituting this robot are equipped with spokelike parts driven by linear actuators. The wheels lie horizontally on the ground and attach to neighboring modules by Velcro. Although no module can move on its own, by using neural oscillators as drivers for the actuators and through the physical coupling between the units, a coordinated global wave of activation can be induced in clusters of more than 30 modules, which leads to forward movement, even though there is no global control.

activity into torques propagating through the individual segments, so that the resulting reactive forces lead to forward movement. An alternative strategy for understanding underwater locomotion attempts to exploit morphology and bio-inspired materials, as demonstrated by a recently built fishlike robot that can swim by simply moving the tail back and forth (*17*). The "trick" that gives rise to lifelike movements in such a strongly underactuated system is the right choice of materials in the caudal fin; materials that allow the "tuning" of the fin shape in a way that seems to optimally distribute the hydrodynamic forces over the fish's body during propulsion and maneuvering (*17*). The tuning is reminiscent of the way in which biological ray-finned fish actively control the curvature of their fins to optimize the transmission of locomotor forces to the aquatic environment, maximizing propulsion while minimizing energy consumption (*37*).

Flying is different from swimming because in addition to the thrust required to move forward, it is necessary to produce sufficient lift to stay in the air (*3*). Despite the impressive mastery of flight by today's technologies, constructing bio-inspired devices capable of nontethered (free) flight remains a challenge. The performance gap between mechanical flapping devices (ornithopters) and their natural analogs is still large (*20*). As for swimming, one potential avenue might be the exploitation of the morphology of bio-inspired materials (*13*). Take an insect wing during hovering flight. Its material properties in terms of resilience, stiffness, and deformability are essential to generate adequate lift in the absence of any forward velocity. For instance, the shape of the wing changes greatly when moving back and forth through the stroke plane (*3*). Although such change in shape could in principle be actively controlled, it is more efficient and faster if the intrinsic material characteristics are exploited and control is outsourced to the morphological and material properties of the wing. An additional advantage of this solution is that the wings can be made much lighter because less actuation is required (Fig. 2E).

Finally, materials can also be exploited for climbing, as beautifully showcased by the uncanny climbing skills of geckos, which can dash up smooth walls and walk across ceilings with great ease. The geckos owe their sticky feet to the structural properties of their toes, which are covered with millions of nanoscale hairlike stalks branching into hundreds of tiny endings (*38*). The use of micropatterned fibrillar dry adhesives inspired by gecko foot morphology is bound to lead to impressive advances in the construction of robots that can climb vertical or inverted surfaces of all kinds (*20*) (Fig. 2F and supporting online material).

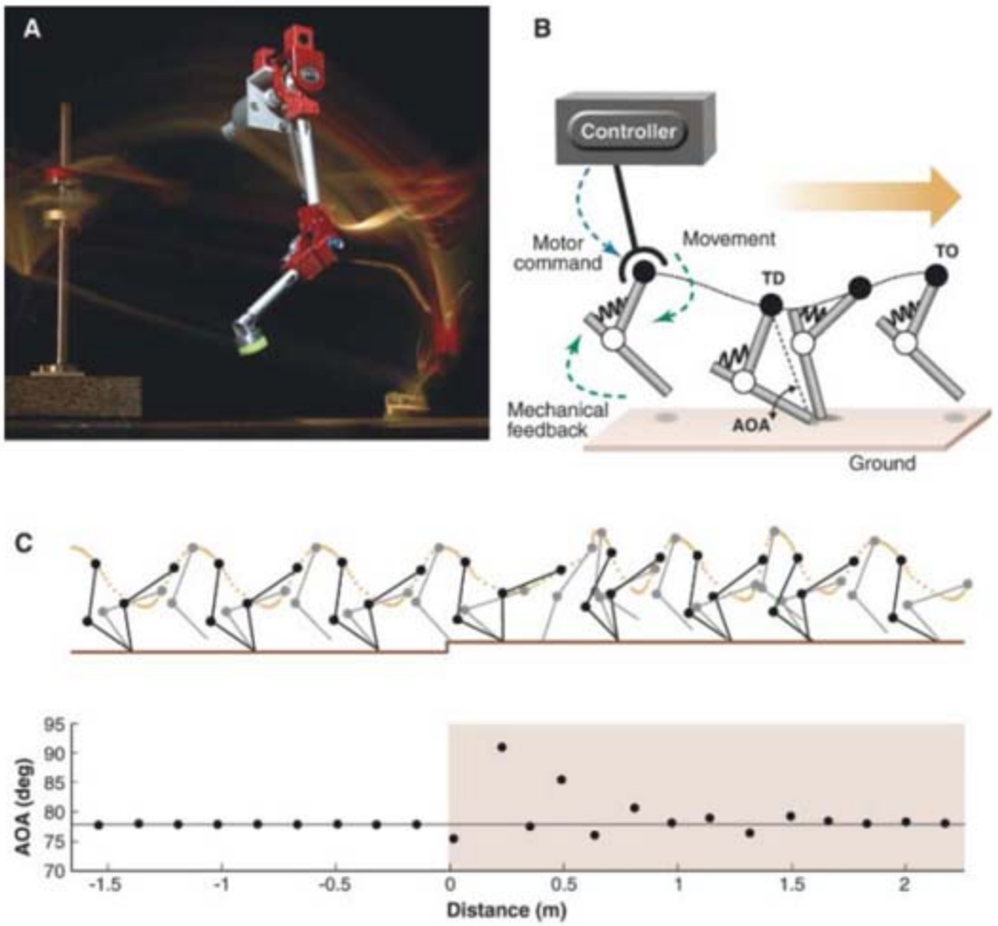

Fig. 3. Self-stabilization. (**A**) Picture of a two-dimensional underactuated monoped hopping robot attached to a central rod with a rotational joint (courtesy of A. Seyfarth and A. Karguth). (**B**) A schematic representation of the hopping robot in the different phases of locomotion: flight, touchdown (TD) [with angle of attack (AOA)], and takeoff (TO). Only the joint depicted by the black circle (hip joint) is actuated, the knee (white circle) is passive, and the lower limb is attached to the upper limb with a simple spring. (**C**) Output of a simulation of the robot. The upper part of the panel shows the trajectory of the model over time as a sequence of stick figures; in the lower part, the angle of attack (the angle at which the leg hits the ground) is plotted. The model exhibits a stable hopping gait with a periodic hip motor oscillation, as indicated by the constant AOA at every step in the left side of the panel. At distance $d = 0$ m, there is a step in the ground that disturbs the robot's movement but to which the robot adapts without the need for any changes in the control. This purely mechanical phenomenon is called self-stabilization. [Figure adapted from (*35*)]

Scaling Up Complexity

Over the past decade or so, with the advent of aging societies and the concurrent advancement of technology, substantial research efforts have been directed toward engineering robots capable of performing a large variety of tasks such as assisting the elderly (by ensuring their quality of life and health care), doing household chores (washing the dishes, cooking dinner, and ironing), helping workers on assembly lines, surveillance, and entertainment. Much progress has been made in the study of basic abilities such as locomotion (*2*, *25*); manipulation (*39*); understanding the surrounding environment, including the recognition of objects, people, and other robots; and social interaction (*23*, *40*, *41*). Because such robots need to operate in environments built for humans, the morphology of choice is humanoid or anthropomorphic.

Humanoid robots often have highly sophisticated sensory-motor systems (*24*, *25*), which implies that they are confronted with the hard problem of processing potentially large amounts of information in real time. Although much research has been conducted on learning in the real world, especially in the fields of artificial intelligence and cognitive robotics (*42*), the tasks and environments, for the most part, have been of relatively limited complexity. One potential reason might be that the size of the search spaces for learning optimal decision policies in realistic scenarios makes the direct transfer of traditional algorithm-based machine-learning techniques to robots not straightforward; more so, if such robots need to operate in real time. Again, exploiting the agent/environment interaction might provide at least part of the solution. As demonstrated by experiments with robots (*4*), the notion of embodiment actively supports and promotes intelligent information processing, greatly simplifying the challenge posed by the need to process large amounts of sensory information (Fig. 4). Consider

a loosely swinging arm: Although the trajectory of the hand is highly complex, the neural control for it is comparatively simple, as coordination is achieved primarily through peripheral mechanical feedback loops and the biomechanical constraints provided by the musculoskeletal system. The behavior that emerges from the synergistic coupling of the arm's morphology, its natural dynamics, and the coupling with the environment yields an effective exploration strategy because it increases the probability that something interesting happens: that the hand encounters and grasps an object and brings it into the visual field or into the mouth. This way, sensory stimulation is not only induced but it also tends to contain information structure, which then strongly simplifies perception and learning (Fig. 4).

Research on bio-inspired cognitive robots that has received considerable attention is imitation learning (*23*, *24*), in which robots learn from humans or other robots. This idea has a special appeal because imitation is a powerful mechanism for reducing the search spaces associated with learning in the real world, which might eventually lead to robots that will need only a minimal amount of programming (*40*). The hope that the imitation problem can soon be resolved has been fueled by the discovery in the mid-1990s of the mirror neuron system (a network of brain areas in the premotor and parietal cortices activated by both recognition and production of object-oriented movements) and its purported link to imitation (*43*). An important challenge in robotic imitation learning is that robots, in spite of their superficial resemblance, have entirely different morphologies from humans. A similar problem is faced by babies trying to imitate adults. Although no generally accepted answer seems to be in sight, it is clear that a biomimetic solution has to take into account the morphological and material constraints to generate the proper imitative dynamics (*24*).

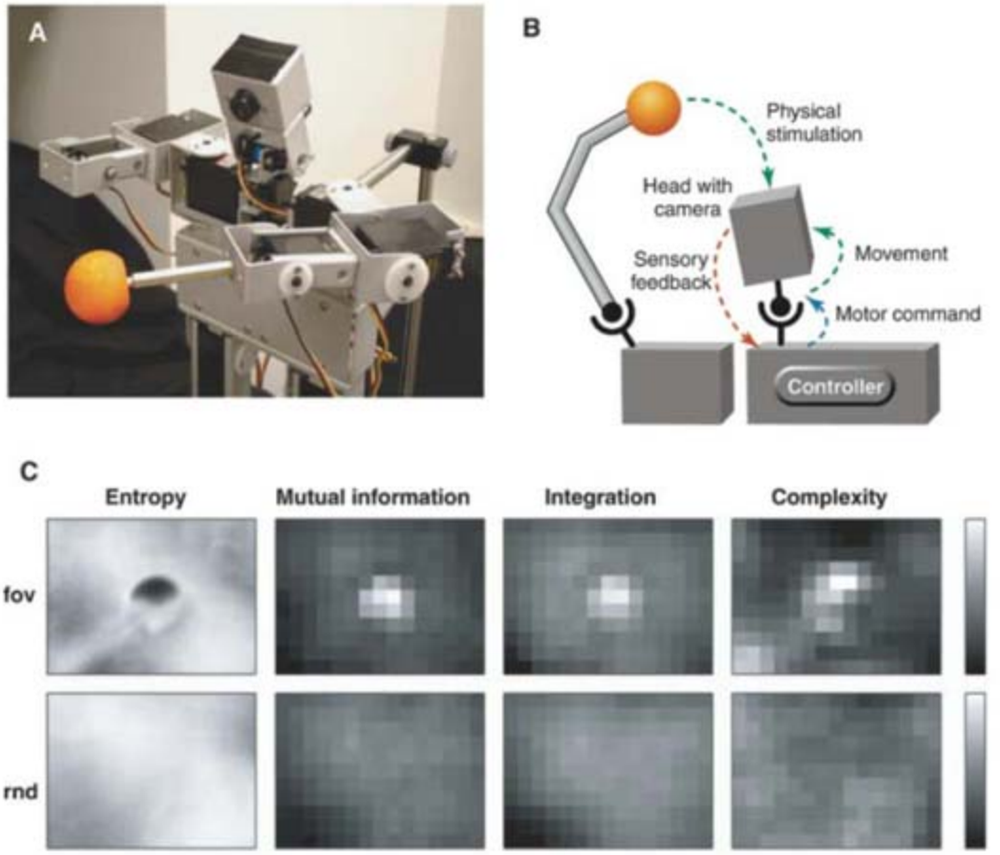

Fig. 4. Information self-structuring. (**A**) Picture of the robot, a small humanoid with a pan-tilt head equipped with a charge-coupled device camera [adapted from (*4*)]. In this experiment, there are two conditions: foveation (fov), where the camera in the robot head tracks an orange ball that moves in the robot's visual field (sensory-motor coupling is undisrupted), and random (rnd), where the movement of the camera is unrelated to the movement of the ball (sensory-motor coupling is disrupted). (**B**) Schematic representation of the experimental setup. The ball is connected to the tip of the most distal link of a robot arm. The arm's movement is preprogrammed and is independent of the head's movement. The movement of the ball results in a displacement of the ball relative to the head and leads to physical stimulation in the head-mounted camera. Sensory feedback is induced, entailing motor commands from the controller. The motor commands entail a movement of the head, which in turn leads to physical stimulation of the camera, thereby inducing information structure. (**C**) Various measures to capture information structure: entropy (the amount of disorder in the system), mutual information (the extent to which the activity of one pixel can be predicted from the combined activities of neighboring pixels), integration (a measure of global coherence), and complexity (a measure that captures global coherence and local variation). The measures are applied to the camera image in the case of the foveation condition (top) and random condition (bottom). As can be seen, there is more information structure in the case of the foveation condition for all measures; for example, the dark region in the center of the entropy panel indicates that entropy is clearly diminished in the center of the visual field, (disorder has been reduced, or in other words, information structure has been induced), which is due to foveation being a sensory-motor coordinated behavior. This example illustrates information self-structuring because through its behavior, the robot is structuring its own sensory input. [Adapted from (*4*)]

Designing Morphologies

Working in parallel with sensory-motor coordination, the specific body morphology (as well as the materials employed) is crucial in shaping the resulting information structure (*2*, *4*). In other words, the design of the controller and that of the morphology are inseparable from each other, because both affect information processing. Yet, although some progress has been made to optimize the design of robot controllers, robot morphology still largely remains a matter of heuristics. In evolutionary robotics, the most widespread approach is to start with a fixed morphology and evolve the robot controller, typically a neural network (*44*). In nature, however, there is never an "empty" organism, but brain (controller) and body (morphology) coevolve. By subjecting the morphology to evolutionary optimization as well, one can not only more fully exploit its power but also make the approach more plausible from a biological perspective. Technically, the robot's morphology is either encoded in the artificial genome in terms of parameters standing for length, diameter, types of joints, and material properties of the basic building blocks that can be used by evolution to build organisms (*45*), or results from a process of ontogenetic development (*46*) of body and brain based on models of genetic regulatory networks (*1*).

If it is indeed the case, as we have argued earlier, that much of the functionality of a robot is due to its particular morphology, then it would be desirable to have robots that, depending on the task at hand, can alter their shape. The term "morphofunctional machines" has been coined to designate devices that can change their functionality not only by a change in control but by modifying their morphology (*47*). Some modular self-reconfigurable robots can "morph," for example, from a snakelike structure into a quadruped walker or vice versa (*7*, *48*) (Fig. 2G), which can be very helpful if the robot needs to move through a narrow space to accomplish its task. Incorporating change of shape into the design considerations is crucial and bears enormous potential for increased adaptivity, versatility, and resilience, an idea that has not been substantially exploited yet.

To date, much of the work in modular robotics is based on macroscopic modules (with sizes ranging from centimeters to tens of centimeters) composed of microprocessors, communication links, sensors, actuators, and mechanical or magnetic docking interfaces (*48*). The size of the modules imposes severe constraints on the kinds of shapes that can be built and the functionality that can be achieved. At micrometer scales, these constraints are less critical, but conventional robotics technology can no longer be applied; it is thus essential to rely on processes of self-assembly—the autonomous organization of patterns or structures with little (or ideally, without) human intervention (*49*). The combination of self-assembly with modular robotics might offer an important strategy for fabricating arbitrary morphologies with specific material properties and for engineering robots displaying truly emergent functionalities (Fig. 2H).

Rather than studying how individual modules aggregate into an organism to perform some functionality, collective robotics investigates how groups of robots cooperate to accomplish a particular task (*50*). Nature provides a wealth of collective phenomena that emerge through processes of self-organization from the local interaction of individual agents (the formation of trails and bridges; sorting, flocking and schooling behaviors; communication; and dominance interactions), which have provided much inspiration for robotics. With some notable exceptions (*50*, *51*), much of the research in collective robotics is still conducted in simulation. Moreover, morphological and material considerations are typically not taken into account.

Self-Replication

The ultimate challenge, self-replicating robots (machines that can autonomously construct a functional copy of themselves), has a lot of romantic appeal but conjures up images of a runaway technological cataclysm. Nonetheless, many approaches to self-replication have been suggested since John von Neumann's seminal work on self-replicating cellular automata almost 60 years ago. Physical self-replicating machines have recently been realized with manually supplied 10-cm cubes that can connect to form arbitrary arrangements (*8*), as well as with electromechanical units randomly floating on an air table, which first grow into a mechanical five-bit string and then self-replicate (*6*). Self-replication is not yet a well-defined subject and different notions of the term "self-replication" exist. Although von Neumann's cellular automaton consisted of more than 150,000 cells, each capable of assuming 29 different states, more recent systems contain just a small number of cells, with fewer states and little reliance on self-organization. It has thus been hypothesized that self-replication is not a clear-cut binary property but a continuous one (*8*). Moreover, we might be more inclined to accept a machine as self-reproducing if it not merely recruits existing modules but assembles them from materials available in its surrounding environment. Although the latter is the goal of some projects (*52*), actual self-reproduction (as observed in biological life) in artificially built systems still needs to be achieved.

Conclusion

Recent work on bio-inspired robots suggests that self-organization and embodiment are powerful concepts in the development of adaptive autonomous systems. Exploiting the dynamics provided by materials and morphological properties as well as the interaction between physical and information processes promises to extend the capabilities of established control-based robot design methodologies. Although bridging the gap between artificial and natural systems will require addressing many conceptual and technological challenges, we believe that a first important step is the abstraction of a set of design principles. Such principles will not only yield a deeper understanding of biological structures and processes but will also guide the construction of novel types of robots of unprecedented diversity and behavioral characteristics. Exciting times are ahead of us.

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Supporting Online Material

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Table S1
References

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REVIEW

Mobile Robots: Motor Challenges and Materials Solutions

John D. Madden

Bolted-down robots labor in our factories, performing the same task over and over again. Where are the robots that run and jump? Equaling human performance is very difficult for many reasons, including the basic challenge of demonstrating motors and transmissions that efficiently match the power per unit mass of muscle. In order to exceed animal agility, new actuators are needed. Materials that change dimension in response to applied voltage, so-called artificial muscle technologies, outperform muscle in most respects and so provide a promising means of improving robots. In the longer term, robots powered by atomically perfect fibers will outrun us all.

In this article, the application of actuator technologies is considered specifically for robots that are humanlike in form. Marc Raibert and his group at Massachusetts Institute of Technology (MIT) showed in the 1980s that robots can walk, run, and do flips (*1*). These robots are not free, however, but rather are attached to their power supplies. The incredible achievements and the limitations of successive lifelike robots provide insight into the challenges of using conventional actuators to drive machines that mimic human form and motion. The focus of this article is on robots and humanoids in particular, but much of the discussion of actuators is relevant to any active mechanical system and particularly those that involve intermittent rather than continuous motion, such as prosthetics, medical devices, valves, locks, and toys.

Combustion Engines: Powerful But Hard to Carry

The power per unit mass achieved in internal combustion engines is 1000 W/kg, about 10 times greater than the continuous power output of our own muscle (*2*). High power makes combustion engines excellent for the propulsion of vehicles, and particularly for highway driving, where abrupt changes in speed or direction are unusual. This power is combined with the long range afforded by the use of gasoline, which has an energy per unit mass that is about 20 times higher than that of a good battery, even after accounting for the ~30% efficiency typical in an internal combustion process. There are two particularly notable challenges to using the combustion engine on a robot, however. The first is that the engine operates best over a narrow range of rotation speeds, providing no torque at all at zero speed. Cars have transmission systems, including clutches and gears, that enable acceleration from a complete stop up to high speed. This transmission is not suited to the abrupt motions required of a robot, such as reaching for an object, then holding it for some time at a fixed position, and then throwing it away. The second challenge is simply carrying the hot, loud, and fuming engine on a robot while operating it efficiently and effectively, with space left for fuel.

Steve Jacobsen and his colleagues have demonstrated particularly impressive use of hydraulics to drive robots (*3*). Hydraulic actuation is a sophisticated version of the system used to drive the shovel on a front-end loader. Jacobsen's hydraulic robotics perform extremely lifelike movements and have been demonstrated in Disney theme park humanoid robots and Jurassic Park dinosaurs. However, these rely on an external power source. The Berkeley Robotics Laboratory has shown that a hydraulic motor can be taken on board (*4*, *5*). Its 75-kg device is not a free-standing robot but rather an exoskeleton with powered ankles, knees, and hips. The robot is attached at the feet and the hips, and it works in parallel with the wearer, allowing an additional 75 kg to be carried. This capability is intended to relieve a foot soldier's burden. The combined hydraulic system, empty fuel tank, valves, actuating pistons, and internal combustion engine exhibit a power-to-mass ratio that is about the same or perhaps a bit lower than that of muscle itself (*6*). Hydraulics are not terribly efficient for walking, which requires high power output only for brief periods of time. For the remainder of the time the system is needlessly shunting fluid. Primarily as a result of this inefficiency, BLEEX expends three times more energy in walking than a human does (*4*). A further drawback is the noise and heat of the combustion engine. The device certainly augments human strength, but so far soldiers are better off building up their own muscle if they can.

One key to reducing weight and increasing efficiency, and thereby making hydraulics more practical, may be to redesign the internal combustion engine to allow for the bursts of power needed during walking, running, or jumping (*7*, *8*). A potential weight-saving measure is to use lightweight pneumatic actuators in place of heavier hydraulic pistons, although this increases the mass of the pump (*9*). Either way, it is very hard to beat muscle.

Electric Motors: Jogging But Not Sprinting

Electric motors are attractive because they feature high continuous power per unit mass [up to 300 W/kg when using rare earth magnets (*10*) and twice that when actively cooled (*11*)] and high efficiency (can be >90%) (*2*). They are also relatively quiet and generate high torques at low speeds, making the transmission easier than it is in the combustion engine. Honda's impressive ASIMO is a battery-powered, untethered humanoid robot driven by electric servomotors (*12*–*14*). There is a motor for each of the 34 joints, including arms, legs, hips, hands, feet, head, and fingers. The fast rotary motion of the electric motors (which deliver maximum power at high speed) is converted to slower joint rotation by using a compact reduction system known as a harmonic drive. The drive has the same effect as going into very low gear on a bicycle. This transmission system, however, is heavy, bringing the overall power per unit mass down to or below that of muscle. Honda's latest robot, shown in Fig. 1, is able to do a slow run (6 km/hour, equivalent to a 16-min-mile pace), with both feet leaving the ground simultaneously between steps, clearing the ground by about 3 cm (*13*). It can also do light work, picking up 1 kg (about four coffees) when using both hands. Similar complexity and performance are demonstrated in other battery-powered servomotor-driven robots, including Sony's QRIO robot (*15*, *16*), which is much smaller than ASIMO and was the first to run, and Kawada's HRP-2 (*16*, *17*), which is about the same size as ASIMO but does not run.

Fig. 1. Honda's humanoid robot ASIMO on the run. Reproduced from (*13*) with the permission of the Honda Motor Company.

Why can't ASIMO and the others go faster, jump higher, or carry a larger load? Speed is limited by the peak power output. Peak power requirements triple in the progression from walking to sprinting (*18*), so ASIMO's motors need to be three times heavier to achieve a fast run

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than they do for a moderately paced walk. In a human the size of ASIMO, the peak power at the ankle is about 200 W (*4*). At a sprint pace, the power rises to 700 W (*18*). Factoring in the inefficiency of the transmission, the power needed from an electric motor is more than 1000 W in each ankle. With transmission included, the power density of the motor is roughly halved, so when using a high-performance uncooled electric motor and gearhead the output is 150 W/kg (*10*), resulting in the need for a 6.5-kg motor on each ankle. Imagine the effect on the quadriceps of carrying an extra gallon of milk on each calf during a sprint: The actuator is simply too heavy.

Mammalian skeletal muscle, the form of muscle we use to move our limbs, has a peak power to mass of about 300 W/kg for fast twitch muscle and lower in aerobic forms (*19*). On the basis of the 700 W required at the ankle during sprinting and optimistically assuming fast twitch performance, 2.3 kg or about 2 liters of calf muscle are required. That is a very large calf muscle, particularly for a person the size of ASIMO (54 kg). Nature gets away with significantly smaller muscles. This is achieved by shunting more than 50% of the energy in a stride in to tendon extension, muscle stretching, and flexion of the foot (*18*). The running motion has been likened to the travel of a pogo stick, and the legs each modeled by a spring in series with muscle. This approach is being mimicked in robotics by inserting springs in series with actuators (*20*) and has been used in several bipedal robots (*9*). In time these may be able to match our own mechanical performance, particularly if metal springs are avoided (the small strains of metals make them low in energy density compared to tendons and rubbers).

Can the electric motors used in robots be improved? The Lorentz force used to drive these motors produces a force that is proportional to current. Current is limited by the heat generated due to resistive losses. Power output can be doubled by adding cooling. One means of improving ASIMO's performance is to add a water circulation system that enables perspiration. In expending 1 kW of energy continuously (a strenuous activity level in a human), little more than 1.5 liters of water per hour would be evaporated. The addition of water cooling is not trivial because it adds complexity, weight, and cost, but making robots that drink to keep cool should dramatically improve agility.

Batteries, hybrids, or fuel cells? ASIMO has a 51.8-V lithium ion battery pack, which can sustain it for 1 hour and takes 4 hours to recharge. Humans can continue for days on their reserves. Our fat, when combined with oxygen, generates enough adenosine triphosphate (ATP) (*21*) (the molecule used to power muscle and other processes) to provide 15 MJ/kg, 30 times more usable energy than the same mass of lithium ion battery. At present ASIMO, with its image and voice recognition abilities, can act as a receptionist, sitting plugged in between making small deliveries or after guiding visitors to their meetings. How can endurance be improved?

Some reduction in energy expenditure may be possible. HRP-2 runs its 11-kg batteries down in about 1 hour, corresponding to an average power expenditure of about 300 W. A person walking at a moderate pace burns about 3.3 W/kg of body mass, a 220-W expenditure for someone weighing the same as the robot (58 kg). The comparison suggests that there are opportunities to reduce power consumption in robots, but what is really needed is a high energy density storage method.

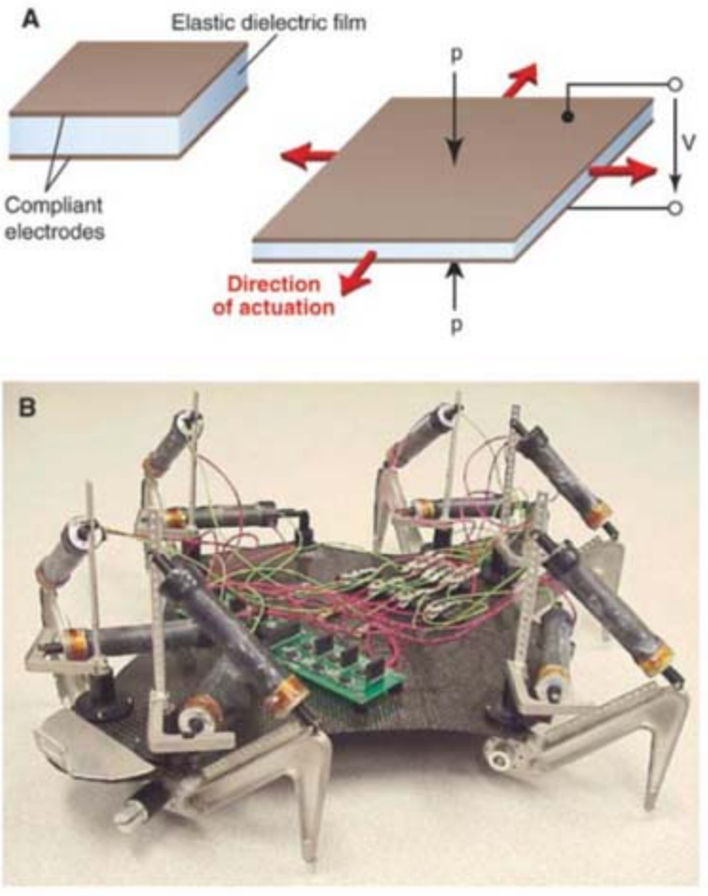

Fig. 2. (**A**) Mechanism of actuation of dielectric elastomers (*21*) and (**B**) SRI's FLEX 2 six-legged robot operating with sheets of dielectric elastomers rolled around a spring to form tubes. Two spring rolls drive each leg. Figure reproduced from (*29*) with permission of the SPIE.

One option is to create the robotic version of a hybrid car. A portable combustion engine driving a 1-kW generator weighs about 15 kg including fuel for up to 8 hours. The effective energy density of the fuel plus the generator over 8 hours is about five times better than that of a battery, but still about five times worse than storing energy as fat. The key to matching fat is to make the motor smaller and lighter. In the long run, the development of turbine generators on a chip could solve the energy challenge. These are millimeter-scale turbine blades, combustion chambers, and electric generators microfabricated in silicon. Fuel-driven microfabricated turbines exhibit power densities that are more than 100 times larger than those in traditional combustion engines, making their size negligible compared to the stored volume of fuel and thus enabling a 20-fold longer running time than is possible with batteries (*22*). Some fabrication challenges remain, however, before these devices are fully demonstrated.

Fuel cells are a promising option but are not sufficiently developed. A commercial portable hydrogen fuel cell (*23*) can provide the same power output per unit mass as the portable gas generator, but the space required is larger because of the fuel volume needed, making it more cumbersome.

Muscle: hard to surpass. The skeletal muscle used to actuate our limbs (*24*–*26*) is a beautifully refined linear actuator, typically capable of contracting by 20% of its length. This large linear contraction is transmitted to bones via tendons, creating a torque about joints that in turn rotates limbs. Cycle life is high, reaching more than 1 billion activations in the heart. The source of energy is chemical and, as with fossil fuels and hydrogen, is very high in energy density in large measure because oxygen is freely available in the atmosphere. The ~45% energy conversion efficiency between ATP and mechanical work is not as good as in a high revving electric motor but is better than that of the combustion engine. A special feature of muscle is that it can selectively activate subsets of fibers within a single muscle. It is also capable of changing stiffness by a factor of 5, a characteristic made possible in part by muscle's ratchetlike actions at the molecular scale. When the ratcheting mechanism is released, the stiffness drops. These properties enable us to grade force depending on load, thereby increasing efficiency and improving control. Imagine trying to catch a baseball with your arms completely stiff or totally relaxed. In the first case, it would

bounce out of your glove before you could grasp it, and in the second the ball would move right through you. The same property enables us to cushion our landing when jumping from a height.

So why not use muscle in robots? Muscle operates optimally when associated with a circulation system that provides oxygen, glucose, and nutrients and can carry away heat, CO_2, and other waste. It also has relatively fine control from nerves that enable rate, force, and speed control. Additionally, the digestive and circulatory systems provide amino acids that enable muscle to build up, repair itself, and regenerate, allowing it to adapt to demand and to last a lifetime. Our technology is not yet ready to interface with such a complex system.

Artificial Muscle

Many materials have been investigated as candidates for artificial muscle (*26*–*28*), including gels that swell and contract by more than 100% in response to changes in pH and temperature; shape memory alloys, whose change in crystal structure with changes in temperature or applied magnetic field produce relative changes in length of up to 10% at high loads; intrinsically conducting polymers that charge and discharge like batteries and swell or contract by about 8% in the process; ionically conductive polymers in which ions and solvent are shuttled from one side of the material to another, producing a bending motion; and liquid crystals, whose change in alignment with temperature or electric field leads to displacements. The two most immediately promising technologies are dielectric elastomers and relaxor ferroelectric polymers. Both are electric field–driven, and they feature high work per unit volume [reaching ~1 J/cm^3, compared to 0.04 J/cm^3 in muscle (*26*)]. The high work density compared with muscle means that less volume and mass are needed (because densities are similar to that of muscle), enabling lighter and thus more agile devices. The relatively good coupling between the electrical input energy and the mechanical work performed (20% to 90%) enables them to operate with efficiencies that are comparable to or better than that of muscle. Dielectric elastomers in particular are ripe for application, having been demonstrated in multilegged robots (*29*) (Fig. 2B) and an arm-wrestling device (*30*), as well as being commercially available from the start-up Artificial Muscle Incorporated of Menlo Park, California.

Electrically driven rubber. Dielectric elastomers (*31*) are thin sheets of rubbery materials (typically silicones or acrylics) whose top and bottom surfaces are coated with flexible electrodes, as depicted in Fig. 2A. The devices are capacitors with compliant dielectrics. When the electrodes are charged, the opposite charges on each electrode attract, leading to a reduction in the distance between capacitor plates and an expansion in the plane. The actuator can more than double in length. These materials outperform muscle in nearly every respect but have their limitations.

Strains increase in proportion to the square of the magnitude of the applied field, so ensuring that breakdown occurs only at very high fields (~100 MV/m) is critical. High dielectric strength is achieved by prestretching films by up to 500%. The problem is that maintaining this prestretch requires a mechanical structure that is generally much bigger and heavier than the elastomer film itself (*26*, *32*). This makes the effective work density much lower, similar to that of muscle. The performance may not be good enough for use in humanoid robots because of additional electronics required to produce the high voltages (>1 kV) that are needed. There are materials solutions that help eliminate the need for prestretching, however, and thereby greatly improve performance. Recent work has shown that, by interpenetrating a stiff, cross-linked polymer between the rubbery chains of the elastomer when in a stretched state, much of the extension is maintained when the load is released (*32*). This eliminates the need to prestrain, improving the work density. The actuators can also be fast, with nearly constant amplitude of displacement having been demonstrated at more than 1 kHz in one form of dielectric elastomers. Important challenges in the application of dielectric elastomers to robotics are finding an effective and compact method of generating high voltages and ensuring safety.

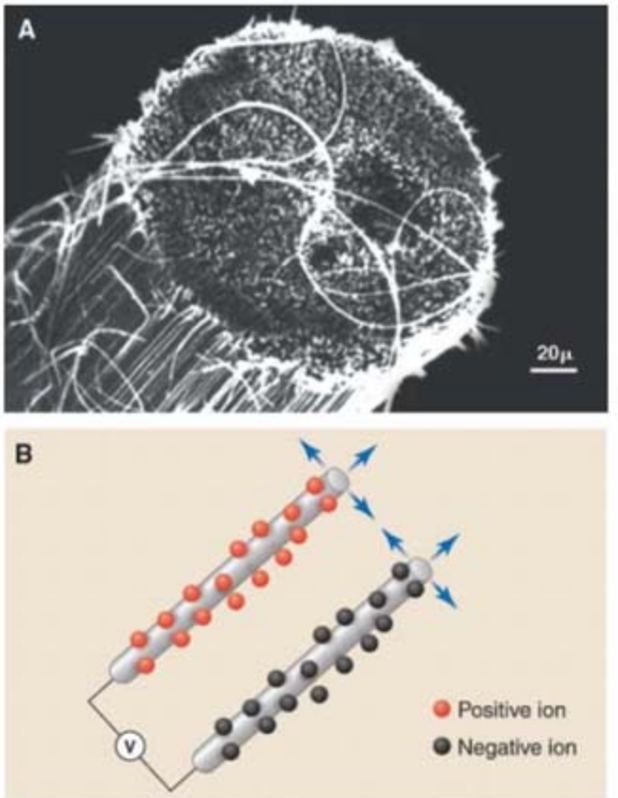

Fig. 3. Actuation of metal nanofibers. (**A**) Scanning electron micrograph of niobium nanofilaments formed by drawing a copper-niobium composite. Reprinted from (*39*). [Copyright 1978 American Institute of Physics] (**B**) A depiction of how such fibers might be actuated, showing two individual fibers to which voltage has been applied through an electrolyte. The charging of the surfaces of the fibers is expected to lead to both expanding relative to their neutral states when charging levels are sufficiently high.

Electric fields that move molecules. Ferroelectric polymers such as polyvinylidene fluoride (PVDF)–based materials generate substantial anisotropic deformations when an electric field is applied (*33*, *34*). The backbone of this polymer is partially fluorinated. The fluorine atoms attract electrons, making the polymer polar. Fields act to change the orientation of the polar groups, altering the conformation of the polymer chains, resulting in displacements. A disadvantage had been the relatively large hysteresis in these materials, similar to that seen in permanent magnets, leading to high switching losses and poor control of displacement. In order to reduce these losses, defects are introduced, which disrupt the formation of large polar domains. In these disordered materials, known as relaxor ferroelectrics, the application of a field to an oriented polymer leads to changes in length of up to 7%. The strain is smaller than that in muscle, making larger mechanical amplification necessary in order to displace limbs. However, stiffness and force per cross-sectional area are higher than in muscle (20 MPa operating stress versus 0.35 MPa in muscle), leading to a much larger work density (about 25 times higher than that of muscle).

Further development is needed in order to determine cycle life and scale the size of these devices up to that needed to run a large robot. As in dielectric elastomers, high voltages are used. There are opportunities to reduce voltages needed in ferroelectric polymers and dielectric elastomers by using thinner layers of materials with a higher dielectric constant and lower stiffness, but these solutions are not as simple as they appear. Increasing dielectric constant can lead to higher stiffness and lower breakdown potential, for example. Another challenge is ensuring that these and other materials can go through the needed number of cycles before failure, because regeneration is

not currently possible. At present, the cycle number is in the tens of millions in dielectric elastomers, adequate for continuous operation once a second 8 hours a day for 1 year.

New Approaches

Can we emulate muscle itself? One approach is to design molecules that undergo reversible shape-changing interactions and to harness these shape changes in order to create musclelike materials. Synthetic motors relying on molecular-scale interactions have been developed (*35*) that could eventually mimic muscle. These include molecules that fold and unfold as a function of applied voltage, single molecule rotary motors, and molecules that slide past each other in response to ion insertion. Creating assemblies of motor proteins is another option. In these cases, a key challenge is not only designing the appropriate molecular-scale interactions but also producing the meso- and microscale structure that enables effective operation on the macroscale. The molecules-to-mechanisms approach has been successfully demonstrated in azobenzene, a molecule that reversibly changes bond shape in response to interactions with photons. Atomic force microscopy measurements show that these molecules exhibit molecular-level length changes in response to light, and similar strains are observed macroscopically (*36*). Light actuation is not always practical. A challenge for the synthetic chemistry and materials communities is to develop molecular mechanisms that are activated directly or indirectly by high energy fuels [e.g., hydrogen and oxygen (*37*)].

An exciting new actuator technology that is currently being explored employs nanotubes. Carbon nanotubes are essentially perfect in their atomic structure. Defects help atoms to slip with respect to each other, causing irreversible deformation. The absence of defects enables these filaments to deform elastically by several percent or more, instead of the 0.1% typical of metals and ceramics. The elastic energies stored in these materials are huge, approaching 10 J/cm^3 in metals, based on elastic strain and modulus. About one-fourth of this energy can readily be extracted (*38*). A sphere 7.5 cm in diameter could contain the active material needed to perform the same work as all of our muscles put together. Our biceps could be replaced by an 8-mm-diameter wire. Such compact muscle would be enormously enabling for robots, making them far lighter and more agile.

In order to extract the energy from nanowires or nanotubes, there needs to be a mechanism of stretching them in the first place. In a cross-bow our muscle provides the stretching, but what can we use to stretch these tiny filaments? Also, if they are to be used in robots, the size needs to be scaled up substantially without producing defects. The stretching can be done electrostatically, as has been shown in carbon nanotubes (*38*), platinum nanoparticles (*39*, *40*), and most recently nanowires (*41*). Charging is achieved by submerging films of these materials into an electrolyte and applying an electrical potential through the solution, as depicted in Fig. 3B. The resulting charging of the surfaces of the nanotubes, wires, and nanoparticles is sufficient to expand these stiff materials because of their high surface area–to–volume ratios.

At present, however, the problem is that the coupling between input electrical energy and output mechanical work is low. Spun nanotube yarns show charge-induced strains of 0.5%, and stresses can exceed 100 MPa. However, the electromechanical coupling is less than 1%. The problem is that a lot of energy is expended stretching the nanotubes, but very little is extracted because the stiffness of the yarn is far lower than that of the individual nanotubes.

How can the coupling and the strain be improved? The conceptually simple but practically challenging answer is by making the macroscopic structures as stiff as the microscopic ones. It has been known for some time that bundles of superstrong nanowires (*42*–*44*), as shown in Fig. 3A, can be as strong as the individual wires from which they are composed. If the bundles can be made porous, then it may be possible to ionically charge them in order to induce deformation.

No actuator technology yet matches the muscular system's combination of high energy density fuel, relatively efficient operation, scaleable force, elastic energy storage, and power output. Developments in transmissions, series elastic elements, and energy storage and generation mechanisms should make it possible to equal muscle's performance using traditional motors. Electric field–driven polymers outperform muscle in most respects but need creative solutions for delivering the electrical power in a safe and compact manner. If the incredible properties of nanofibers can be extended to macroscopic scales in actuators, as has been achieved for passive mechanical structures, then artificial muscle will enable robots to outrun and outjump us all.

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REVIEW

Robotics in Remote and Hostile Environments

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In our continuing quest for knowledge, robots are powerful tools for accessing environments too dangerous or too remote for human exploration. Early systems functioned under close human supervision, effectively limited to executing preprogrammed tasks. However, as exploration moves to regions where communication is ineffective or unviable, robots will need to carry out complex tasks without human supervision. To enable such capabilities, robots are being enhanced by advances ranging from new sensor development to automated mission planning software, distributed robotic control, and more efficient power systems. As robotics technology becomes simultaneously more capable and economically viable, individual robots operated at large expense by teams of experts are increasingly supplemented by teams of robots used cooperatively under minimal human supervision.

The drive to explore is a human quality that has changed our understanding of the world and the universe we inhabit. Today the frontiers of exploration have moved to distant and hostile environments, to which we can travel only at great expense. Visiting the abyssal sea floor requires a sophisticated submersible launched from a ship staffed with highly trained specialists. Venturing as far as Earth orbit requires the resources of a nation. The technical challenges and costs of keeping humans alive in harsh and distant environments have led to an increasing use of robots as proxies. In space, scientific results obtained by unmanned robotic spacecraft are already impressive. Their many discoveries include providing the best evidence that water once ran on the martian surface (*1*), discovering the existence of methane lakes on Titan (*2*), and verifying the runaway greenhouse effect on Venus (*3*). In contrast to deep space, the ocean has been accessible to humans, although only at substantial cost. What robots promise for the ocean sciences is a great reduction in the threshold for access, allowing a much more pervasive presence in the ocean. Already mobile robots are in use in almost every domain in the ocean, from the previously unsurveyed cavities under floating ice shelves (*4*) to the volcanically active mid-ocean ridge system where new sea floor is being formed (*5*).

Deep space and the ocean's interior are often associated with difficulty in communications; consequently, an important measure of a robot's effectiveness there is its ability to function with little or no human supervision. Unless an underwater vehicle is operating within acoustic communication range of an appropriately equipped ship (typically on the order of a few kilometers), the only communication option is to surface and communicate via satellite. Typically, satellite communications options for small vehicles provide bandwidths up to only 10 kilobits per second at sea. This contrasts with communication rates over 10 times higher available to the Mars Exploration Rover (MER) vehicles on the surface of Mars (Fig. 1). However, the round-trip communication time to Mars can be as long at 40 min. For many tasks, introducing such a lag in the control loop is either fatal or debilitating to productivity. Thus the marine environment and the space environment provide a common motivation to endow robotic platforms with greater onboard autonomy.

Autonomous mobile robots used in exploration activities are highly dependent on their ability to sense and respond to their environment. In contrast to a robot in structured settings, such as a factory floor, an exploration robot must accomplish its goals in a previously unmapped environment with unpredictable disturbances and threats. At one time, building a robot that reliably carried out a set of preprogrammed tasks was a technical accomplishment. Today, exploration robots are expected to sense their surroundings and act to avoid problems or improve performance. For example, operational underwater robots are expected to avoid bottom collisions in most circumstances. The more sophisticated their perception of their surroundings, the greater their ability to respond constructively. Consequently, attention is now turning to fielding practical robots capable of replanning their mission in response to changing circumstances while deliberating on how best to satisfy the goals and expectations given to them by human operators.

The technological evolution of exploration robotics is shaped by our understanding of emerging scientific needs. Although space and ocean robotics present many of the same problems, the importance of the ocean to climate prediction on Earth creates additional imperatives for marine robots. The ocean is a large thermal reservoir, and its circulation, determined by winds, Earth's rotation, and variations in temperature and salinity, moves heat from low to high latitudes. Beyond its physical properties, the ocean comprises the largest ecosystem on the planet; although the function of the vast majority of marine organisms is yet to be determined, one known function is to produce approximately half of the oxygen we breathe (*6*). Yet at the same time, the ocean is one of the least-well-observed portions of the planet. Remote sensing techniques examine the sea surface while leaving the bulk of the ocean unobserved. What is emerging is a need for observation systems that are capable of making coordinated measurements in many places at the same time.

Scientific challenges, such as understanding global climate change, are addressed in a highly interdisciplinary environment. A particular robot, or collection of robots, will need to respond to a wide array of scientific goals, which may be intertwined by operational necessities evolving on a short time frame. For example, ocean observing systems composed of large numbers of coordinated observation assets (described later in this paper) serve many investigators, each with their own research agenda. Thus, an emerging model is the use of the Internet to support collaborative frameworks, allowing participants to engage each other in the simultaneous development of scientific understanding and operational plans.

Interplanetary Exploration

Early failures of robotic and launch platforms for space missions in the 1960s helped focus technology development toward making spacecraft hardware robust. Software to run these vehicles for interplanetary exploration has been created with comparably simple command-and-control software both onboard and on Earth. To this day, spacecraft are predominantly controlled with predefined commands that are generated a priori by human controllers and communicated to the vehicle. The control sequence is then executed onboard with limited contextual awareness. In the void of interplanetary space, such lack of situational awareness has a limited impact on the operation of the spacecraft. However, inevitable contact with the environment on planetary surfaces, coupled with round-trip light time delays, have to date implied large numbers of human operators on Earth who are carefully crafting commands to ensure the health and safety of the robot. This

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has often led to less-than-optimal use of these robotic proxies, if not affecting their economic efficiency. For instance, during the nominal mission, the twin MER vehicles were supported around the clock, by upwards of 200 engineers colocated at the Jet Propulsion Laboratory (JPL).

In 1999, NASA's Deep Space 1 spacecraft (DS1) demonstrated a truly autonomous robotic mission capability with the deployment of the Remote Agent artificial intelligence (AI) (*7*, *8*) system. Sixty-five million miles from Earth, it was able to deliberate onboard and demonstrate failure diagnosis and recovery from injected faults. Also onboard was an autonomous navigation (*9*) capability that used pattern recognition to compare images of stars with a known star catalog to triangulate the spacecraft's position during its interplanetary cruise. This coalesced a long-espoused view by researchers in the fields of robotics and AI: the sense-plan-act (SPA) model, in which the robot senses its environment, decides whether an a priori (or newly) generated course of action is appropriate, and based on that determination, actuates its sensors to observe, sample, or move in its environment. More recently, in laboratory tests, science-based autonomous operations have demonstrated detection and tracking of pre-specified events of scientific interest (*10*), coupling pattern recognition with onboard deliberation on wheeled robots. This points to an interesting and necessary convergence between the fields of autonomy, machine learning, and robotics to tackle real-world problems of scientific interest in detecting and tracking episodic events, such as dust devils on the martian surface.

On DS1, the onboard autonomy requirements necessitated the coordinated use of pattern matching with deliberation and command execution. In performing a trajectory correction, the Remote Agent would throttle down the engine, request the attitude control system to execute the turn while keeping the solar panels aligned to the Sun, take pictures of an appropriate star to validate its course correction using the AutoNav system and onboard star catalogs, and then throttle up to resume its cruise. It would do so by averaging out image jitters while damping the turn, ensuring that Sun-angle constraints and the uncertainty of turn times, as well as mitigation for camera component failures, were taken into consideration during command execution. During the course of the experiment, failures were injected to demonstrate the system could gracefully recover and continue without undue human intervention, demonstrating the impact such techniques have in mission operations as well as in dealing with events of scientific opportunity. This was an early and dramatic demonstration to prove that AI techniques are finally maturing while dealing with real-world complexity. They have done so after decades of fundamental research in knowledge representation, automated reasoning, and computational search [see (*11*) for a comprehensive view of fundamental AI techniques], which are central to deliberation and AI as a whole.

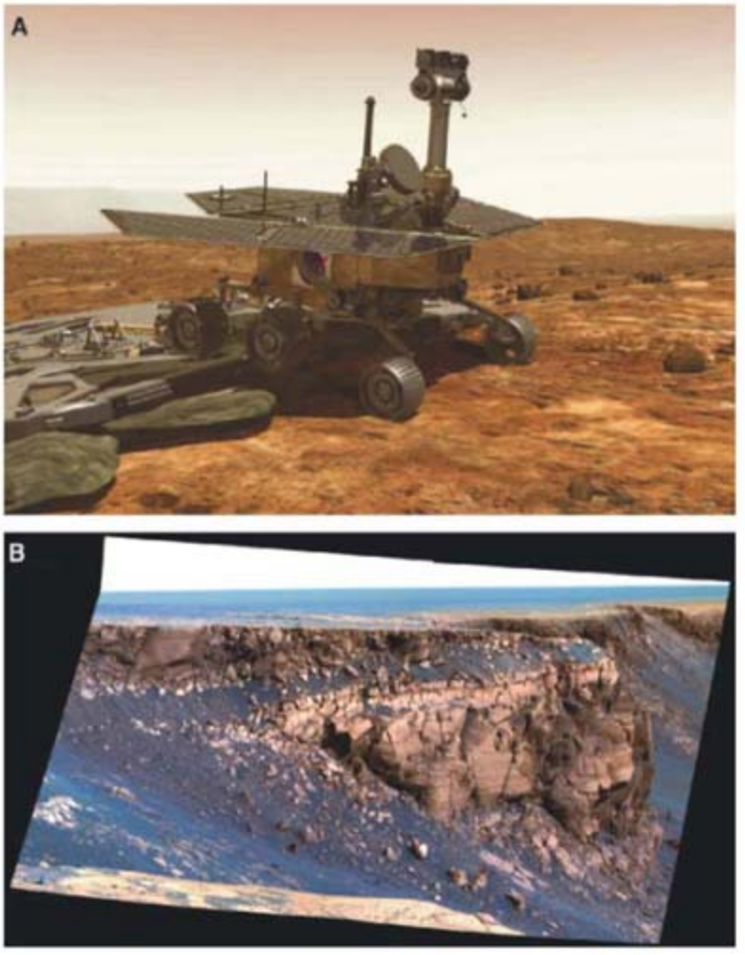

Fig. 1. Robots now roam the surface of a distant planet, exercising increasing levels of autonomy. (**A**) A MER vehicle leaving the lander platform to begin its exploration of Mars. [Credit courtesy NASA/JPL-Caltech] (**B**) False-color image of a promontory jutting out from the walls of Victoria Crater, Mars, which is being explored by NASA's MER Opportunity rover. This image was taken by Opportunity's panoramic camera on sol 1167 (6 May 2007). It is presented in false color to accentuate differences in surface materials. [Image credit: NASA/JPL/Cornell]

However, because of the perception of risk, the adoption of onboard deliberation techniques by the operations and science communities has proceeded slowly. One variation of the SPA paradigm that is increasingly popular is that of mixed-initiative systems (*12*), in which humans are aided by and in turn guide the formulation of plans by a computer. Such mixed-mode interaction uses the substantial cognitive capabilities of humans together with the intrinsic capability of computers to deal with numerical computation when aiding robots in decision-making. The command and control of the MER vehicles, for instance, uses techniques originally used for onboard deliberation but now used on the ground at JPL in the Mixed-Initiative Activity Plan GENerator (MAPGEN) (*13*) for planning science and engineering activities. Although this system does not close real-time sensing loops, it allows scientists on Earth to decide what science activities to plan by specifying constraints on their observations while abstracting out and dealing with the engineering details of the rover hardware situated remotely on Mars. Early proving tests at JPL showed a 20% increase in the quantity of science data returned while sustaining the quality when using such mixed-initiative techniques over a purely manual approach. This led to confidence in deploying the MAPGEN tool set, which to date is the longest-running AI program in a mission-critical role in the space domain. Such modalities hold promise for engineered systems where the complexity of the environment, if not of the platform itself, currently necessitates human/computer interaction, another key area of research in AI and robotics.

Software engineering techniques have progressed substantially to exploit hardware breakthroughs in computation and sensing. Lower-level functionalities are no longer where AI and robotics researchers are spending the bulk of their efforts in attempting to make robots more effective; rather, higher-level decision-making capabilities stemming from better understanding of robotic control, coupled with progress in AI search and automated reasoning techniques, are pushing the boundary of how robots deal with the real world.

Observing Earth's Ocean

In contrast to space robotics, which is shaped by the high costs of launch and the complete absence of opportunity for human intervention should problems be encountered, robotics in the ocean sciences has been a grassroots affair. Most efforts start with comparatively small budgets and only gradually develop into larger programs. Developers of undersea robots often accompanied their

systems to sea, sometimes working in rough weather or in the middle of icebound oceans. The development of autonomous marine robots for science started in the 1960s with the Self-Propelled Underwater Research Vehicle (SPURV) (*14*). SPURV was the first autonomous underwater vehicle, or AUV, and was used to measure horizontal variability in the ocean, a property that is hard to characterize from a ship. By the early 1990s, over 56 different AUVs were described in the published literature, although almost all were demonstration vehicles rather than operational platforms, and many had never been successfully operated (*15*). In the past half-decade, AUVs have seen adoption by not just the science community but also the military and the oil and gas industy. The result is a growing number of commercial companies that make AUVs and their specialized subsystems. As a consequence, the state of AUV technology and AUV capabilities is evolving rapidly (Fig. 2).

Early applications of AUV's started in some of the most remote environments, such as the deep ocean and the Arctic; as the platforms have matured, they're increasingly used even in easily accessible locations and in a variety of roles. The efficiency and stability of AUVs as both sonar and imaging platforms have encouraged a growing use of AUVs for producing high-resolution maps of the deep sea floor (*16*, *17*) as well as photomosaics (*18*) to support a wide range of science interests (Fig. 3). The Autonomous Benthic Explorer (ABE) uses progressive search strategies to find hydrothermal vents, in which the vehicle starts with a wide area search for a neutrally buoyant hydrothermal vent plume and then progresses to finding the more localized buoyant plume and sea floor structure of hydrothermal vents (*19*). At high latitudes, AUVs have been used to make measurements under ice, measuring heat flux (*20*, *21*) and distributions of biological populations (*22*), both key observations for understanding the current rapid rate of change of Arctic climate and ecosystems. AUVs are also being used for more routine operations, such as in shallow coastal environments (*23*).

Limitations on energy storage are a fundamental driver in AUV design, and different applications motivate quite different types of vehicles. To achieve long endurances or ranges, designers have two options: to make the vehicle very large and thus capable of carrying large quantities of batteries, or to make the vehicle very slow and low-powered. In many cases, factors such as the size and power consumption of the scientific sensors force the vehicle to be large. Vehicles such as Autosub (*24*), ABE (*25*), Hugin (*26*), and Dorado (*27*) are all examples of larger systems used to carry more sophisticated ocean science payloads such as mapping sonar or diverse collections of sensors for characterizing physical, chemical, optical, and biological properties of seawater. Gliders (*28*–*30*) are an example of small underwater platforms with long endurance. They are a cousin to the profiling floats already present in large numbers in the ocean (*31*). Gliders move by changing their buoyancy and using lifting surfaces, such as wings, to translate vertical into horizontal motion, instead of the propellers used by most other AUVs.

The success of autonomous platforms has encouraged the development of sensors that capitalize on and complement the availability of lower-cost methods of observing the ocean. Although sensors to measure physical properties such as temperature, salinity, and current have been available for many years, many research laboratories are creating sensors for the chemistry (*32*) and biology of the ocean. For example, the introduction of instrumentation for determining nitrate concentrations (*33*) allows AUVs to characterize nutrient availability in situ, where it previously could be determined only by laboratory analysis of samples taken from ships. A range of techniques for identifying small organisms is being developed as well, including systems that optically image organisms and use computer recognition to classify them (*34*).

The need to observe and understand the internal weather of the ocean and the changing composition of ocean ecosystems at ever-higher spatial and temporal resolutions has motivated the creation of observing systems using fleets of AUVs. Such observations provide a density of ocean observation that has not been possible before, useful for a range of purposes, including the improvement of understanding and parameterization of ocean processes important to climate models. The Autonomous Ocean Sampling Network (AOSN) (*35*, *36*) uses fleets of small, highly capable mobile systems as a coordinated sampling network, linked to predictive assimilative ocean models to observe and predict ocean processes. AOSN is both enabled by, and has motivated, small, long-endurance underwater platforms but also relies on larger vehicles carrying chemical and biological sensors. By combining vehicles optimized around different observational objectives, a more complete observing system can be created. Integral to AOSN is the coupling of observations to real-time physics-based oceanographic models, which both synthesize disparate measurements into a realization of the environment and provide a predictive tool. The need to opti-

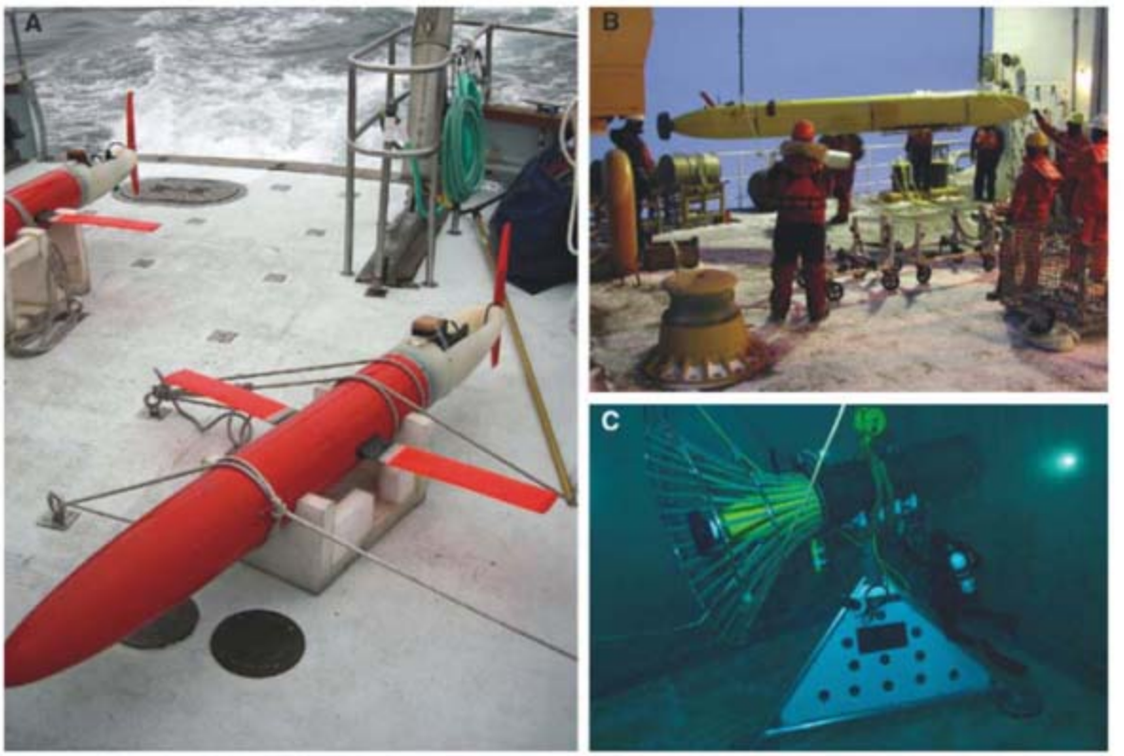

Fig. 2. (**A**) An underwater glider on the deck of a boat, ready for deployment. Gliders can make simple measurements of ocean properties such as temperature and salinity for months at a time, traveling at a speed of about 25 cm/s. (**B**) A Dorado AUV, capable of carrying complex payloads such as mapping sonar and comprehensive suites for analyzing the physical, chemical, and biological properties of seawater at speeds of 1.5 m/s. This image shows the AUV after recovery by the U.S. Coast Guard cutter *Healy*, in the icepack north of Svalbard. (**C**) A docking station with a Dorado vehicle captured in the docking cone, shown in testing before deployment. This device was connected to a cabled observatory, allowing Internet connectivity with the vehicle and the charging of vehicle batteries (*46*).

mize the performance of such complex collections of observation elements and models motivates study of survey optimization and adaptive sampling (*37*–*39*). Thus, the success of AUV technology is leading to the creation of ocean observing systems composed of diverse assemblies of underwater robots.

Trends for the Future

Exploration robotics is transforming from an activity in which individual robots are deployed for fixed periods and operated by teams of specialists to a pervasive activity operating continuously by distributed multidisciplinary teams. This will be driven by technological advances in four key areas: sensing, autonomy, development of supporting infrastructure, and collaborative software systems. In the oceans, the energy limitations of existing systems will be surmounted by new strategies for extracting power from the environment and using scientific power and communications infrastructure being installed in the ocean.

A robot's capability is a strong function of the sophistication of its capability to sense its environment. For example, a sensing system that processes camera imagery to identify and locate rocks makes rock sampling an easier task for onboard control software. However, a scientist will want the robot to be selective in choosing rocks. Thus a more sophisticated system might classify rocks, allowing their prioritization for sampling. In essence, the decision-making process is made much simpler if sensing systems can provide the right information. Autonomous robots are already able to build perception-based semantic networks (*40*–*42*) that allow them to label and localize objects in structured surroundings; the models that leverage such capabilities will migrate from meticulously handcrafted entities to ones increasingly learned by the robot during exploration. Thus the rapid advances of the sensor community will be augmented by advances from the artificial intelligence community to provide information to decision-making software in a much more relevant form.

Advances in autonomy will not only be driven by better sensing, but it in turn will drive and increasingly be driven by online learning, novel methods for representing and reasoning about uncertainty, and software engineering capabilities for verification and validation. Machine-learning techniques are already learning the evolution of simple natural phenomena (*43*); the next logical step will be to build models of the environmental changes, allowing robots to make more informed (and potentially optimal) decisions while executing mission plans, thus making them more adaptive. Robots will then be able to deal with unstructured environments and hostile conditions on planetary surfaces while simultaneously deliberating about their mission goals and real and potential failure conditions. Those in the ocean will be able to "sniff" out and follow gradients toward their sources, whether they be freshwater plumes, harmful algal blooms, or effluents from mid-ocean hydrothermal vents, characterizing and sampling their environment and generating high-resolution bathymetric data while determining their own location and ensuring their health and safety, all without any human supervision. Yet none of these advances in robotic autonomy is likely without increased trust in how implemented software is working or is expected to work. Validation (is this the right system for the job) and verification (is the system built right) techniques before long will certify the viability of the software for the tasks for which it was designed and deployed.

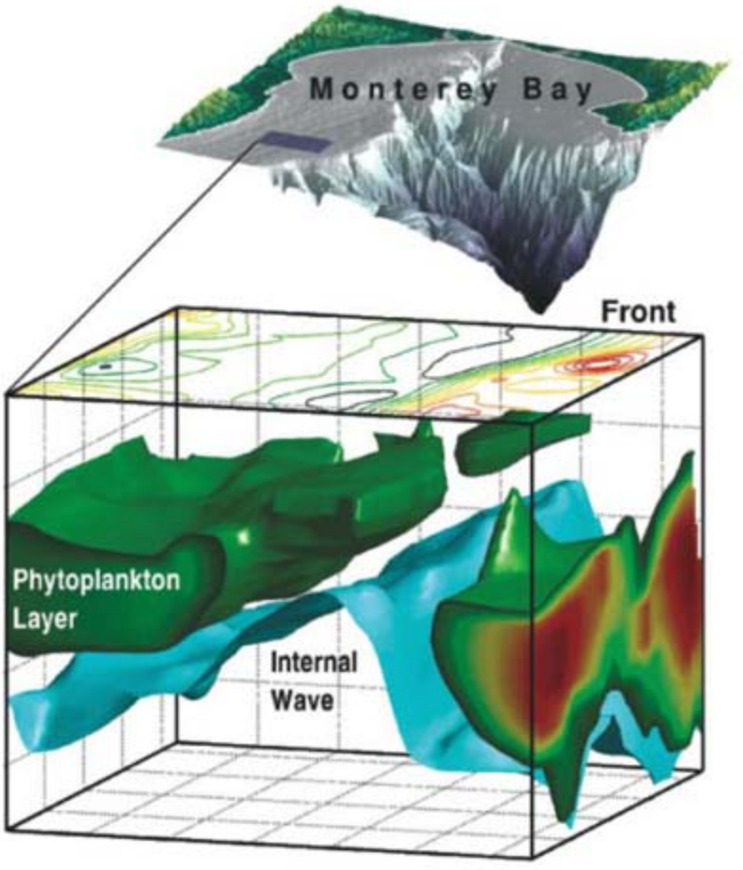

Fig. 3. A three-dimensional image of the interaction of physical and biological processes, as mapped by an Odyssey AUV off the coast of California (*52*). The green volumes show a phytoplankton layer, detected by its chlorophyll fluorescence. The underlying cyan surface shows deflection of the constant-density surface by an internal wave, interrupting the phytoplankton layer. To accomplish this survey, the AUV moved in a sawtooth pattern across the survey area while profiling vertically. The volume shown is 6.5 by 2.5 km in horizontal extent and 23 m in depth.

Mobile robots are particularly restricted by their ability to generate or store energy. Over the past several decades, batteries have become safer, and secondary batteries can be recharged more often, but only incremental changes in energy density have been realized. Space robots have mitigated battery limitations by using solar panels to greatly increase the lifetimes of space missions. Efforts are under way to build solar-powered AUVs (*44*) and AUVs that draw their energy from thermal gradients in the ocean (*30*). Efforts targeting the creation of untethered buoys that can hold their position against prevailing currents and winds are attempting to extract energy from wind and waves. Tapping the chemical energy stored in sediments on the sea floor is the goal of yet other efforts, which are creating microbial fuel cells (*45*) producing useful power levels. Complementing these are development programs that have created docking systems that allow an AUV to connect to an underwater structure to establish power and communication links (*46*, *47*). Using a docking station, an AUV's deployment would no longer be limited by the energy storage capacity of the vehicle.

As the productivity of fielded systems improves, operational demands on science teams increase dramatically, motivating the development of software to facilitate interacting with robotic exploration systems. Typically, researchers from geographically diverse institutions move to a common location. However, this is viable only when missions are short and science teams are small. In the case of MER, the planned 90-martian solar day (sol) duration of the surface exploration mission brought upwards of 400 scientists and engineers from the United States and Europe to JPL. With the extension of MER, now in its fourth year of operation, the mission is operated by 50 personnel at JPL, supported by a distributed science team (*48*). Thus the success of robotic exploration creates the need for collaborative portals that allow distributed research teams to efficiently interpret data and collaborate on the development of new operational plans. An early example of such a collaborative infrastructure was the Collaborative Ocean Observatory Portal (COOP), designed for the Monterey Bay 2006 field program (*49*). COOP leveraged data from a diverse array of observational assets and models to create synoptic views of oceanographic fields and

fluxes over an area of approximately 1000 km^2, provided forums for the discussion of results, provided direct access to data and models, and included a highly successful framework for building consensus on planning remote asset deployments. In operation, the system relayed information from platforms and sensors to shore in near real time, where it could be assimilated into three independent ocean models. The collaborative tools now emerging are lowering the barriers to participation in scientific exploration and will be key to the success of future observatories.

Finally, as robots become more pervasive, emerging trends include the creation of supporting infrastructure and the increasing specialization of individual robots. The benefits of this evolution are visible in both deep space and the ocean. The MER vehicles now relay their information to Earth via a series of Mars orbiters, which allows the MER platforms to transmit more data while simultaneously preserving energy for science activities (*50*). The net result is the creation of a valuable infrastructure for communication, increasing the scientific utility of the MER mission. In the ocean, the benefits of using specialized platforms with complementary capabilities are integral to the development of a distributed observing system such as AOSN. One of the largest initiatives under way in the ocean sciences is the creation of an infrastructure to distribute power and communications to the sea floor (*51*). These sea-floor observatories will use combinations of moorings and sea-floor cables to distribute power to nodes in the deep ocean. Although the diverse approaches to ocean observing have roots in different research communities and have developed different technologies, their complementary capabilities are likely to coalesce to enable a more comprehensive robotic presence in the ocean. In space and beneath the waves, we see a trend toward more specialized robots, with more effective sensing capabilities resulting in more autonomy, accessible via collaborative portals through which researchers can engage with each other, the data, and their robot surrogates.

Conclusions

Robots are gaining acceptance in both space and ocean exploration. In the oceans, the need to more comprehensively understand Earth's climate and the great difficulty of making observations in the ocean's interior have created the need for persistent large-scale observing systems composed of heterogeneous mixes of robots. In space, the closely choreographed operation of robots by a large number of operators on Earth is slowly giving way to a model in which humans supervise increasingly capable robots. In both domains, the need to carry out complex tasks at distances, with either delayed or absent communications, precludes human control of every action. Full autonomy is becoming a necessity. Vehicles that seem like science fiction, capable of simple self-repair and dealing with the complexities of the hazardous environment around them, may well provide a more permanent and pervasive presence in the distant reaches of our planet's oceans as well as in the solar system in the coming decades.

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PERSPECTIVE

Learning in and from Brain-Based Devices

Gerald M. Edelman

Biologically based mobile devices have been constructed that differ from robots based on artificial intelligence. These brain-based devices (BBDs) contain simulated brains that autonomously categorize signals from the environment without a priori instruction. Two such BBDs, Darwin VII and Darwin X, are described here. Darwin VII recognizes objects and links categories to behavior through instrumental conditioning. Darwin X puts together the "what," "when," and "where" from cues in the environment into an episodic memory that allows it to find a desired target. Although these BBDs are designed to provide insights into how the brain works, their principles may find uses in building hybrid machines. These machines would combine the learning ability of BBDs with explicitly programmed control systems.

In the last several decades, great progress has been made in describing how the separate components of the nervous system function. Less progress has been made in obtaining a global picture of higher brain functions such as learning and memory. This picture must take into account that the brain is embodied and that the body and brain act together in a real-world environment. Here, I briefly describe a synthetic approach to elucidating how integrated nervous systems operate by constructing brain-based devices (BBDs). These devices differ fundamentally from robots that are controlled by built-in artificial intelligence consisting of explicit programmed instructions (*1*). Instead, like real animals, BBDs must learn autonomously to categorize signals from the environment without a priori instruction.

BBDs are constructed according to a procedure called synthetic neural modeling (*2*). In such an approach a detailed brain is simulated in a computer and controls a mobile platform containing a variety of sensors and motor elements. In modeling the properties of real brains, efforts are made to simulate vertebrate neuronal components, neuroanatomy, and dynamics in detail (Fig. 1A). As a result of the exploration of a real world environment by a BBD, the BBD develops adaptive behavior through processes mimicking those underlying learning in animals. In its close mimicry of vertebrate neural systems, the BBD approach stands in contrast to other more functionally oriented neurorobotic models (*3*).

To bring out the differences from programmed robots, I shall describe the composition and behavior of two BBDs called Darwin VII and Darwin X. These devices are named after the great biologist Charles Darwin to emphasize the fact that their brains learn by selection from a repertoire of many different simulated neural circuits and do not depend on explicitly programmed instructions.

Darwin VII (Fig. 1A) has a mobile base fitted with a video camera for vision, a pair of microphones for audition (contained in two plastic cups), and a gripper device that can grab steel blocks in its environment, each painted with either stripes or blobs. The BBD's brain consists of a visual system, an auditory system, a "taste" system in the gripper (which measures the conductance of a gripped block), a motor system capable of triggering movement, and a value system.

The value system in the BBD's brain signals the salience of environmental cues and leads to rewarding or aversive responses that enable the device's learning behavior to be adaptive. The simulated neurons underlying these systems are linked in circuits modeled on known anatomy. The connections within these neural circuits (synapses) can change in their strength after receiving sensory signals. The patterns of these changes are unique to each individual BBD because they reflect that device's past behavior. Darwin VII's complete nervous system contains about 20,000 simulated neuronal units linked together by 450,000 synaptic connections [see supporting online material (SOM)]. As the BBD was exploring the environment, the complex responses of these units to signals from the environment were modeled in a large computer cluster and were radioed to Darwin VII to direct motor activity. Consequent changes in the sensory inputs to the device were sent back to the simulated brain in a dynamic fashion, allowing smooth movement in real time (*4*).

The basic modes of behavior of Darwin VII consist of visual exploration and tracking, gripping and tasting, and two innate reflex responses: appetitive and aversive. Figure 1A shows Darwin VII approaching a steel block with stripes (detected by the camera) that was arbitrarily constructed to have good taste (high conductance). Gripping the block sent appetitive signals to the value system in the brain. Blocks containing blobs had low conductance (bad taste) and sent aversive signals to that system.

In a series of conditioning experiments, Darwin VII autonomously explored its environment and learned to associate the taste value of the blocks with their visual patterns. Appetitive and aversive responses were initially triggered by taste, but after about 10 encounters with a population containing both types of block, the BBD's responses were triggered by vision alone. More than 90% of the time, after training, the BBD continued to grip and taste striped blocks but actually backed away from blocks with blobs (Fig. 1B).

This autonomous development of patterned behavior by instrumental or operant conditioning requires sophisticated visual anatomy (see SOM for details). The inset of Fig. 1A shows the patterns of activity of four simulated areas as the BBD moves. The upper left square illustrates a pattern of activity in an early visual brain area that responds in a striped pattern to a striped block. The upper right square shows a firing pattern of neuronal units in an integrative higher visual region corresponding to the inferotemporal area of the brain, an area in animal brains that responds differentially to different object categories. This particular pattern is dependent on the BBD's history of encounters with striped blocks and thus is unique to that history (an identical Darwin VII with a different history would have its own characteristic but equally correlative pattern). The colored areas in the lower right-hand square signal positive value responses as appetitive taste, even before gripping. Appetitive responses in brain circuits prompt motor action, resulting in approach and grabbing of the block. The lower left-hand square would signal aversive responses, but none were experienced in this sequence. If Darwin VII encountered the block with a blob pattern (see block at bottom of main panel), aversive activity would be signaled in that square.

A critical feature of such a BBD is that, after a period of training and behavior, the activity of all neuronal units, synapses, and circuits can be recorded and examined in detail. This cannot be achieved in experiments on living animals. As I shall mention later, the patterns obtained from this type of exhaustive analysis can be of great value in analyzing brain function. To extend such analyses, experiments can be performed to examine experience-dependent changes in the BBD's perceptual categorization and learning. These changes have included learning followed by reversal learning; i.e., learning after switching appetitive and aversive correlations.

Darwin VII's memory after learning did not reflect sequences of events. Given this limitation, my colleagues and I asked whether we could model long-term episodic memory in a BBD. This

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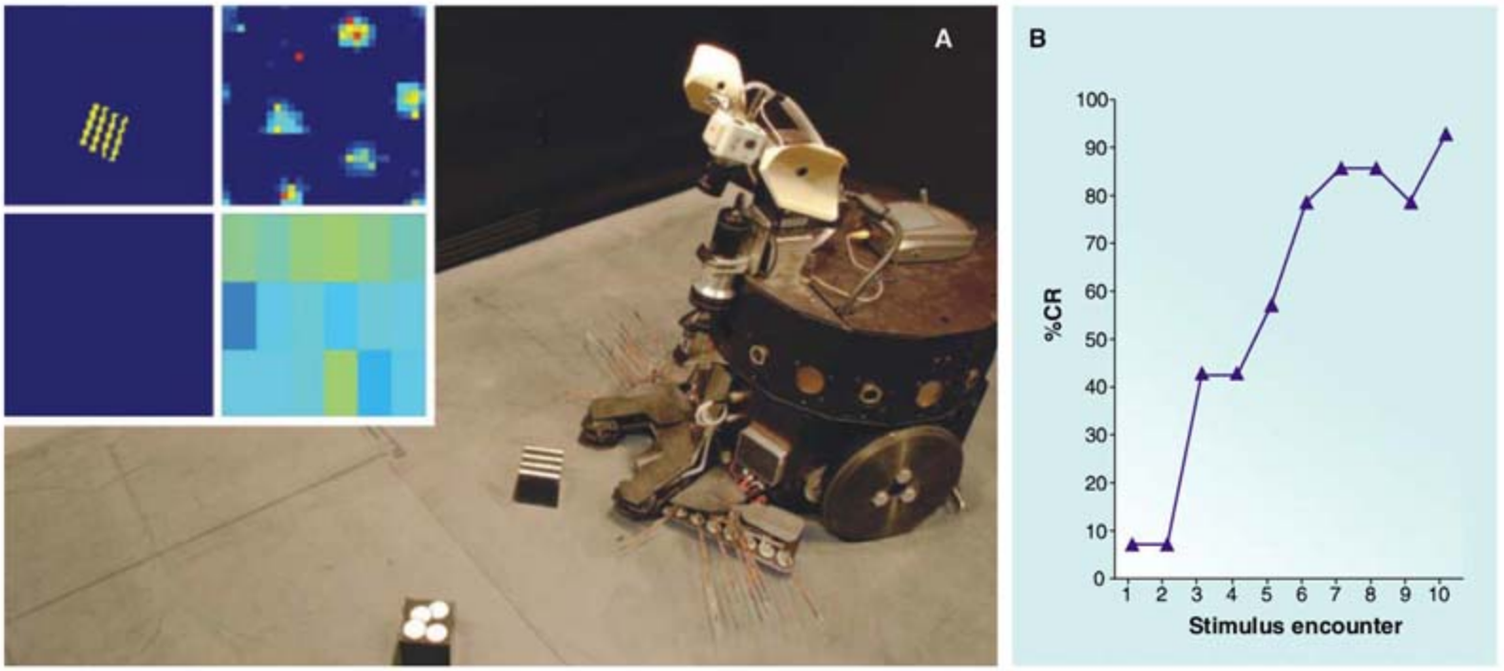

Fig. 1. (**A**) Darwin VII approaching a striped block after training. The mobile device is equipped with a charge-coupled device camera for vision, microphones for hearing, and a gripper capable of sensing levels of conductance for taste. (Inset) Patterns of activity of four simulated areas as the BBD moves. Upper left, activity in an early visual brain area responding in a striped pattern to a striped block; upper right, firing pattern of neuronal units in an integrative higher visual region corresponding to the inferotemporal area of the brain; lower right, colored areas signal positive value responses as appetitive taste, even before gripping; lower left, aversive responses (none in this sequence). See text for further details. (**B**) Percentage of correct responses (CRs) as a function of stimulus block encounters during learning trials for visual conditioning.

memory of sequences of events in real time is known to depend critically on a brain structure called the hippocampus. Patients who have lesions of the hippocampus bilaterally can remember episodic events in their early lives, but after the occurrence of the lesion can no longer convert short-term memory into long-term episodic memory. In rats, the hippocampus is also known to be essential for the successful integration of cues allowing navigation to a goal in a remembered environment. One test of this capability is provided by the so-called Morris water maze. A rat is placed in a tank of milky water that has a hidden platform below the surface. The animal will traverse various paths until it locates the platform, where it will then rest. Having seen various cues on the wall of its surroundings during its traverse, the rat possesses episodic recall and can subsequently locate the hidden platform directly upon being placed again in any part of the tank. Lesions of the hippocampus compromise this ability.

We decided to model the hippocampus in the brain of a BBD called Darwin X (*5*). This device was then tested in a dry version of the maze (Fig. 2A). An enclosure was constructed with a black floor and walls. Each wall had differently colored paper strips of varying widths that could act as visual cues. At one location we placed a "hidden" platform. The visual system of Darwin X could not detect the platform, but the BBD was equipped with an infrared (IR) detector that would respond only when the device was directly over the platform. That detector's signal then triggered a positive value response. This response is analogous to that of a rat in a water maze sensing a solid platform under its feet.

Darwin X was given the task of finding the hidden platform, and after 8 to 10 traverses during which it detected different cues on the

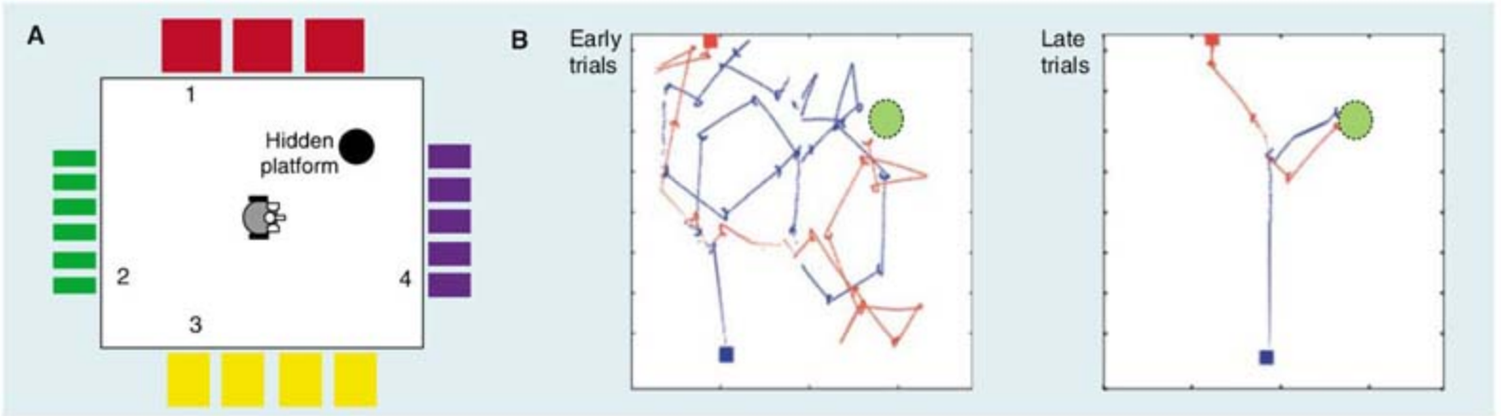

Fig. 2. (**A**) Schematic layout of the enclosure used for the hidden-platform task. The enclosure measured 16 by 14 feet, with black walls and flooring. Pieces of differently colored paper of varying widths (to act as different cues) were hung on each of the walls. A hidden circular platform—24 inches in diameter and made of reflective black paper—was placed in the center of a quadrant in the enclosure. Each trial began in one of four starting locations (numbers 1 to 4 in the diagram). (**B**) Behavioral performance in the hidden-platform task indicated by trajectories of a subject during the training paradigm. Green circles denote the location of the platform during the training trials, red and blue squares denote the starting locations, and red and blue lines indicate trajectories during individual early and late trials. The late trials showed more or less direct paths, regardless of the starting point. [Adapted from (*4*)]

wall, it would directly go to the platform from any starting point (Fig. 2B). Indeed, if the hidden platform was removed, a trained Darwin X would, like a trained rat, concentrate its searches in the area previously occupied by the platform.

An analysis of the neural responses of the simulated hippocampus revealed that they closely resembled those in the living animal. The simulated brain of Darwin X had 50 different neural areas, 90,000 neuronal units, and 1.4 million synaptic connections. We could record the response of every neuronal unit and connection in the BBD, a procedure not possible in a living animal. After training, we could pick any neuronal unit that sent signals to the motor system and trace back the types and strengths of connection of all neuronal units that caused the firing activity of this reference unit. An examination of the resultant backtrace network revealed a very large number of different possible paths and circuits leading to the firing of a single chosen reference unit (5). Thus, the system showed degeneracy, the property according to which different structures can lead to the same response or output (6). This observation suggests that degeneracy might also be a common property of the neural networks of living animals. By providing these insights, synthetic neural modeling has enhanced our efforts to understand how the human brain works.

In addition to these fundamental issues, there is a practical implication for the field of robotics of the work on BBDs. It is now possible to construct hybrid machines incorporating the principles of BBDs together with engineering principles that rely on programmed instructions. An example is a robotic soccer-playing device that we constructed on the platform of a Segway Human Transporter at the suggestion of the Defense Advanced Research Program Agency. This device was designed to play together with a human teammate in the 2005 U.S. Open Robocup Championship in Atlanta. It had a video camera for recognizing objects on the field (balls, teammates, goals, etc.), IR sensors and a laser range finder to detect the ball, and a ball-capturing and -kicking device. The Segway platform's behavior was guided by a neural simulation in an on-board computer that received inputs from the various sensors and generated motor signals to the Segway's wheels. In addition to the simulated brain containing neuronal units, it had a set of programs (like a conventional robot) guiding some of its reflex responses. Playing against an excellent team from Carnegie Mellon University that used a Segway device based on artificial intelligence, our team won all five games (7). We attribute this largely to the ability of our Segway device to learn by experience before the game.

We expect that, although BBDs were initially designed to further our understanding of the human brain, their principles may complement those currently used in various engineering approaches. Clearly, there are several other areas of robotics that, to some degree, have taken account of biological principles or that may contribute to the effectiveness of hybrid designs. This prompts a detailed comparison of our work with two growing fields of robotics. The first, evolutionary robotics (8), views robots as autonomous artificial organisms that develop skills by selection after interacting with their environment. The second, probabilistic robotics (9), is concerned with perception and control by robots in the face of uncertainty.

BBD design is still in its early stages. It will be greatly enhanced by the development of small, very powerful computers that are capable of direct placement on board the platforms of such devices. A far-off goal of BBD design is the development of a conscious artifact (10). Although machine consciousness (11) is at best a remote prospect, the fact that we can build BBDs that are capable of perceptual categorization with sophisticated memory systems provides an initial basis for what a decade ago would have been considered science fiction.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5853/1103/DC1
SOM Text
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Hurricane Katrina's Carbon Footprint on U.S. Gulf Coast Forests

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Forests recovering from land use, the expansion of woody vegetation, and other ecological processes produce a net terrestrial CO_2 sink of ~1 to 2 Pg C year^{-1} (*1*). The United States contributes an estimated 0.30 to 0.58 Pg C year^{-1} to this global sink, with 26 to 33% being actively sequestered in forest trees (*2*). Changes in the strength and sign of this sink over the coming decades are difficult to predict, but as secondary forests mature the sink strength is likely to diminish (*3*). Another process that can diminish the terrestrial carbon sink is an increase in disturbance frequency and intensity (*4*), which transfers biomass from live to dead respiring pools and shifts the stem distribution toward smaller average tree size and lower biomass stocks (*5*). Here, we quantify hurricane Katrina's carbon impact on Gulf Coast forests by using a synthetic approach combining detailed field investigations, remote sensing image analyses, and empirically based models for regional scaling.

To develop spatially explicit maps of hurricane forest impacts, we used spectral mixture analysis (SMA) (*6*, *7*) on Landsat imagery to quantify per-pixel fractional abundance of green vegetation (GV), nonphotosynthetic vegetation (NPV: wood, dead vegetation, and surface litter), soil, and shade for seasonally matched Landsat 5 images captured before and after the storm. The fractional change in NPV (ΔNPV) from 2003 to 2006 provided a quantitative measure of the change in dead vegetation associated with Katrina. A subset for the Pearl River basin was stratified by ΔNPV to generate disturbance classes, and forest inventory plots were randomly established across the entire ΔNPV disturbance gradient (Fig. 1A). In each plot, tree mortality and damage, species composition, and biomass loss were quantified.

A strong correlation between Landsat-derived ΔNPV and field-measured tree mortality and damage (fig. S1) enabled development of tree mortality and damage maps from the Landsat imagery. Next, a second scaling function was generated by comparing Landsat- and MODIS-derived ΔNPV. With the high temporal frequency and large spatial dimension of MODIS imagery, the Landsat-MODIS scaling provided an assessment of hurricane disturbance across the entire impact region (Fig. 1B). To carry out this scaling, we generated distribution functions for stem density and tree biomass from our forest inventory plots and additional U.S. Forest Service data. A Monte Carlo model was developed to estimate stem density, biomass distribution, mortality, and damage for all forested pixels in the MODIS scene (fig. S2) affected by Katrina. Statistically evaluated minimum and maximum values for key model parameters were used to estimate prediction error intervals (table S1). Nominal runs of the model predicted mortality and severe structural damage to 320 million large [>10-cm diameter at breast height (DBH)] trees (range from 290 to 350) with a total biomass loss of 105 Tg C (1 Pg = 1000 Tg) (range from 92 to 112), an amount equivalent to 50 to 140% of the net annual U.S. carbon sink in forest trees (*2*).

Methods for calculating the contribution of forest trees to the terrestrial carbon sink include summing tree recruitment and growth and subtracting mortality (*2*). Although carbon in coarse woody debris (CWD) from tree mortality and damage is not immediately respired to the atmosphere, this CWD pulse largely represents committed future CO_2 emissions (*5*). Although subsequent forest recovery from disturbance can offset CO_2 emissions from decomposing CWD, a sustained increase in disturbance intensity or frequency (or both) will reduce forest tree carbon stocks and ultimately cause ecosystems to act as a net CO_2 source (*8*). If a warming climate causes more extreme events and greater storm intensity (*4*), elevated forest tree mortality will increase CWD production, resulting in higher ecosystem respiration and a potentially important positive feedback with elevated atmospheric CO_2.

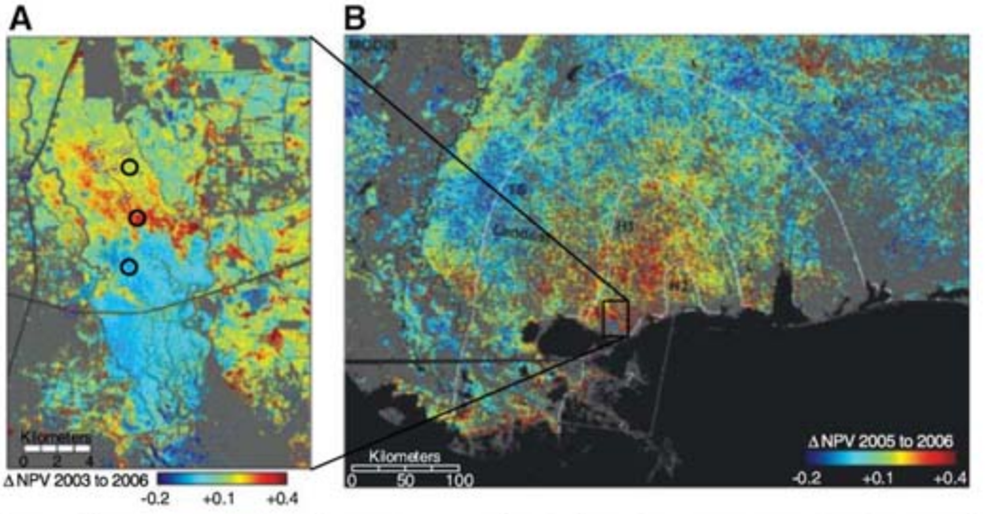

Fig. 1. (**A**) Pre- to posthurricane change in the NPV fraction (ΔNPV) on a Landsat 5 subset for the Pearl River basin (Louisiana-Mississippi state line) provided a quantitative measure of disturbance intensity. By using this map, we established forest inventory plots (white markers) across the disturbance gradient. Open black markers represent (top) moderately resistant, infrequently flooded, bottomland hardwood forest; (middle) minimally resistant, frequently flooded, bottomland hardwood forest; and (bottom) highly resistant, flooded, cypress-tupelo swamp forest. (**B**) MODIS-derived ΔNPV from 2005–2006 provided regional estimates of tree mortality and biomass loss across the entire impact region. Isotachs (white lines) represent tropical storm (TS), category 1 (H1), and category 2 (H2) wind fields (*9*).

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Supporting Online Material

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The Genomic Landscapes of Human Breast and Colorectal Cancers

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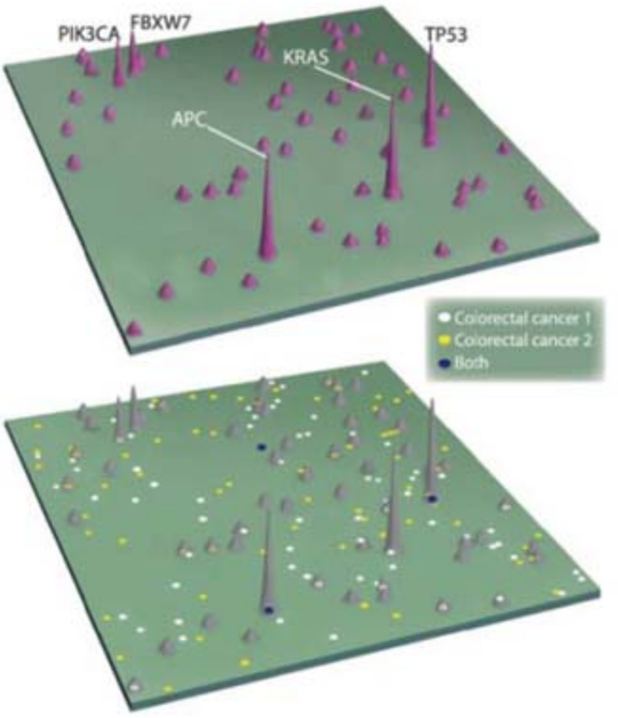
AUTHORS' SUMMARY

How many genes are mutated in a human tumor? Answering this question would have seemed like science fiction just a decade ago. However, as a result of advances in technology, we have been able to answer this question in breast and colorectal cancers: There are ~80 DNA mutations that alter amino acids in a typical cancer. Examining the overall distribution of these mutations in different cancers of the same type leads to a new view of cancer genome landscapes: They are composed of a handful of commonly mutated gene "mountains" but are dominated by a much larger number of infrequently mutated gene "hills."

The current study expands upon previous work (*1*) and includes analysis of the sequences of 20,857 transcripts from 18,191 human genes, including the great majority of those that encode proteins. The genes were sequenced in 11 breast and 11 colorectal cancers. Any gene that was mutated in the tumor but not in normal tissue from the same patient was analyzed in 24 additional tumors. Selected genes were further analyzed in another 96 colorectal cancers to better define their mutation frequency and aid subsequent bioinformatic analyses.



A two-dimensional map of genes mutated in colorectal cancers, in which a few gene "mountains" are mutated in a large proportion of tumors while most "hills" are mutated infrequently. The mutations in two individual tumors are indicated on the lower map. Note that most mutations are outside hills or mountains and may be harmless.

Statistical analyses suggested that most of the ~80 mutations in an individual tumor were harmless and that <15 were likely to be responsible for driving the initiation, progression, or maintenance of the tumor. Though the numbers of mutant genes in breast and colorectal cancers were similar, the particular genes that were mutated were quite different, as were the type of mutations found. For example, mutations converting 5′-CpG to 5′-TpG were much more frequent in colorectal than in breast cancers, indicating differences in mutagen exposure or DNA-repair processes.

The mutational landscapes of cancers can be shown on a map on which each gene is represented at a single point (see figure for the landscape for colorectal cancers). The heights of the peaks reflect the mutation frequency of each gene. A few gene "mountains" are mutated in a large proportion of tumors; most genes are mutated in <5% of tumors and are represented as "hills" in the figure. In the lower panel, the mutated genes in two colorectal tumors are indicated by differently colored dots. The mutated genes in the two tumors overlap to only a small extent. These differences are likely to be the basis for the wide variations in tumor behavior and responsiveness to therapy.

Historically, the focus of cancer research has been on the gene mountains, in part because they were the only alterations that could be identified with available technologies. However, our data show that the vast majority of mutations in cancers do not occur in such mountains. This new view of cancer is consistent with the idea that a large number of mutations, each associated with a small fitness advantage, drive tumor progression (*2*). It is the "hills" and not the "mountains" that dominate the cancer genome landscape.

Are these landscapes hopelessly complex? The large number of "hills" actually reflects alterations in a much smaller number of cell signaling pathways. Indeed, pathways rather than individual genes appear to govern the course of tumorigenesis (*3*). Accordingly, we devised methods to classify mutant genes into commonly altered pathways. Disruption of a pathway by mutation in any one of its genetic components would presumably lead to similar changes in growth. The <15 driver mutations in an individual tumor likely reflect alterations in a similar number of pathways.

Sequencing alone cannot definitively determine whether a specific gene "hill" actually contributes to tumor formation. We therefore used various bioinformatic and structural analyses to help determine which were pathogenic. Integration with functional studies will also be essential; indeed, several of the candidate cancer genes identified in our study have been independently implicated in tumorigenesis through functional studies reported by others.

In sum, our results make it clear that it is now "easy" to identify the genetic alterations in cancers on a genome-wide scale. It is much more difficult to elucidate the precise role of these alterations in tumorigenesis. The compendium of genetic changes in individual tumors provides new opportunities for individualized diagnosis and treatment of cancer. Taking advantage of these opportunities is the major challenge for the future.

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FULL-LENGTH ARTICLE

Human cancer is caused by the accumulation of mutations in oncogenes and tumor suppressor genes. To catalog the genetic changes that occur during tumorigenesis, we isolated DNA from 11 breast and 11 colorectal tumors and determined the sequences of the genes in the Reference Sequence database in these samples. Based on analysis of exons representing 20,857 transcripts from 18,191 genes, we conclude that the genomic landscapes of breast and colorectal cancers are composed of a handful of commonly mutated gene "mountains" and a much larger number of gene "hills" that are mutated at low frequency. We describe statistical and bioinformatic tools that may help identify mutations with a role in tumorigenesis. These results have implications for understanding the nature and heterogeneity of human cancers and for using personal genomics for tumor diagnosis and therapy.

Discovery of the genes mutated in human cancer has provided key insights into the mechanisms underlying tumorigenesis and has proven useful for the design of a new generation of targeted approaches for clinical intervention (*1*). With the determination of the human genome sequence and improvements in sequencing and bioinformatic technologies, systematic analyses of genetic alterations in human cancers have become possible (*2*–*4*).

Using such large-scale approaches, we recently studied the genomes of breast and colorectal cancers by determining the sequence of the Consensus Coding Sequence (CCDS) genes, a collection of the best-annotated protein-coding genes (*5*). In this study, we have extended these analyses to include examination of all of the Reference Sequence (RefSeq) genes. The RefSeq database is a comprehensive, nonredundant collection of annotated gene sequences that represents a consolidation of gene information from all major gene databases (*6*). The RefSeq database is believed to include the great majority of human gene sequences and represents the gold standard in the field.

Sequencing strategy. The first step in our approach was the design of primers that would permit polymerase chain reaction (PCR)-based amplification and analysis of coding exons in the RefSeq database. Of the 20,857 transcripts in the RefSeq database (representing 18,191 distinct genes), 14,661 transcripts were included in the CCDS set. These CCDS genes were in general not evaluated again; the only exceptions were a small subset in which particular regions of interest had been difficult to amplify and for these, new PCR primers were designed. For the remaining 6196 Refseq transcripts, 125,624 primers were designed and used to amplify the coding exons. The entire list of primers used to amplify the exons of the RefSeq genes (including the CCDS genes) is provided in table S1.

The primers were used to PCR-amplify and sequence the DNA from 11 breast and 11 colorectal cancers, as well as DNA from matched normal tissues of two patients. The samples used for this analysis were the same as those used in the previous study of CCDS genes (*5*). The sequence data from this Discovery Screen were assembled and evaluated using stringent quality criteria (*7*), resulting in successful analysis of 93% of targeted amplicons. We used bioinformatic and experimental strategies to distinguish germline variants and artifacts of PCR or sequencing from true somatic mutations (fig. S1). Genetic alterations found in the two normal samples and those present in SNP databases were removed and sequence traces of the remaining potential alterations were visually inspected to remove false-positive calls in the automated analysis. After these steps, the amplicons of the remaining alterations were re-amplified from the tumor DNA (to ensure reproducibility) and from DNA of matched normal tissue (to remove unannotated germline variants). Finally, the putative somatic mutations were examined "in silico" (by computer analysis) to ensure that the alterations did not occur as a result of mistargeted amplification of related regions of the genome (*7*).

To further evaluate the genes with somatic mutations in the Discovery Screen, we determined their sequence in a Validation Screen of 24 additional samples of the same tumor type in which the mutation was originally identified. Methods similar to those noted above were used to exclude germline variants, PCR and sequencing artifacts, and alterations due to mistargeted amplification of related genomic regions. Amplicons with putative somatic mutations were re-amplified in DNA from the tumor and from matched normal tissues to determine whether the alterations were truly somatic.

Somatic mutations. Combining the data from the current analysis with those previously obtained in CCDS genes, we found that 1718 genes (9.4% of the 18,191 genes analyzed) had at least one nonsilent mutation in either a breast or colorectal cancer (Table 1 and table S3). The great majority of alterations were single-base substitutions (92.7%), with 81.9% resulting in missense changes, 6.5% resulting in stop codons, and 4.3% resulting in alterations of splice sites or untranslated regions immediately adjacent to the start and stop codons (Table 1). The remaining somatic mutations were insertions, deletions, or duplications (7.3%). The mutation spectrum of colorectal cancers differed from that of breast cancers, and these spectra were similar to those observed in the previous CCDS study and in other analyses (*4*, *5*). In this study, we analyzed the nature of the nonsynonymous mutations in more detail and found a very large excess of C to T transitions at 5′-CpG-3′ in colorectal cancers, representing 19 times as many as expected from the representation of 5′-CpG-3′ sites in the coding regions of the genome. Similarly, there was a marked excess of G to C transversions at 5′-GpA-3′ sites in breast cancers, representing 4.5 times as many as expected (*7*).

Passenger mutation rates. The somatic mutations found in cancers are either "drivers" or "passengers" (*4*). Driver mutations are causally involved in the neoplastic process and are positively selected for during tumorigenesis. Passenger mutations provide no positive or negative selective advantage to the tumor but are retained by chance during repeated rounds of cell division and clonal expansion.

We used two independent methods to estimate the passenger mutation rates in the analyzed cancers. First, we evaluated 23.8 Mb of chromosome 8 in 11 colorectal cancer samples similar to those used in the Discovery Screen. This was performed with high-density oligonucleotide microarrays containing every possible single-base pair substitution. The tumors used for this analysis each had only one allele of chromosome 8 [i.e., they showed loss of heterozygosity (LOH)], rendering the detection of sequence alterations sensitive and reliable. A total of 151 somatic mutations were identified in 262 Mb of tumor DNA, and all but one of these were located in noncoding regions. Thus, there were a total of 0.6 noncoding mutations per Mb analyzed (95%

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Table 1. Summary of somatic mutations. UTR, untranslated region. ND, not determined because synonymous mutations were not evaluated in the RefSeq genes analyzed in (*5*).

Tumor type	Screen	Gene set	Mutated genes	Coding changes						Noncoding changes	Total mutations
				Missense	Nonsense	Insertion	Deletion	Duplication	Synonymous	Splice site or UTR	
Colorectal cancers	Discovery	This study	325	237	14	0	8	0	93	12	364
		All RefSeq	848	722	48	4	27	18	ND	30	942
	Validation	This study	88	81	9	1	2	2	30	6	131
		All RefSeq	183	197	34	4	14	5	ND	15	299
Breast cancers	Discovery	This study	460	304	26	2	28	1	131	14	506
		All RefSeq	1137	909	64	5	78	3	ND	53	1243
	Validation	This study	62	52	3	0	3	0	19	2	79
		All RefSeq	167	153	11	2	15	2	ND	7	209

confidence interval: 0.52 to 0.64 mutations/Mb). Because only one copy of chromosome 8 was analyzed in these studies, the noncoding mutation rate per diploid genome was inferred to be 1.2 mutations/Mb. We then performed detailed LOH analyses of the 11 tumors used in the Discovery Screen using 317,503 polymorphisms. An average of 16% of polymorphic alleles showed LOH. It is known from studies of human genetic variation that the frequency of nonsynonymous (amino acid–changing) mutations is approximately half that of mutations in noncoding regions (*8*, *9*). After correcting for LOH and the difference in mutation rates between noncoding and nonsynonymous mutations, these analyses result in an estimated passenger mutation rate of 0.55 nonsynonymous mutations per Mb of tumor DNA in colorectal cancers (*7*). We consider this a minimum estimate as the ratio of mutations in noncoding regions to nonsynonymous mutations in coding regions is likely to be higher in the germ line than in tumors because of greater negative selection for mutations in coding regions in the germ line. Although we have not directly measured mutation rates in noncoding sequences in breast cancers, Stephens *et al.* have estimated that the rate of nonsynonymous mutations in breast cancers is 0.33 per Mb, and we used this as our minimum estimate for this tumor type (*10*).

Estimates of the passenger mutation rates were also obtained through the quantification of synonymous (silent) missense mutations in this study. Because most synonymous changes are expected to be biologically inert and thereby not selected for or against during tumorigenesis, such changes can be used as a tool to estimate passenger mutation rates (*11*). The analysis of synonymous mutations provided two estimates of the nonsynonymous mutation rate (*7*). One estimate was based on the ratio of nonsynonymous to synonymous mutations observed in the human germ line (*8*, *9*). The second estimate was derived by calculating the expected ratio of nonsynonymous to synonymous changes after accounting for codon usage of RefSeq genes and the different mutation spectra observed in colorectal and breast cancers. We considered this estimate to be a maximum because it did not take into account that nonsynonymous mutations that retard cell growth will be selected against during tumorigenesis.

Evaluating mutated genes. The mutational data obtained can be used to identify candidate cancer genes (*CAN*-genes) that are most likely to be drivers and are therefore most worthy of further investigation. In this study, we considered a gene to be a *CAN*-gene if it harbored at least one nonsynonymous mutation in both the Discovery and Validation Screens and if the total number of mutations per nucleotide sequenced exceeded a minimum threshold (*7*). Using these criteria, we identified a total of 280 *CAN*-genes, equally distributed between colorectal and breast cancers (table S4, A and B, respectively). The 280 *CAN*-genes listed in table S4, A and B, included most of the 191 *CAN*-genes identified in Sjöblom *et al.* (*5*) but differed by virtue of the inclusion of 114 new *CAN*-genes identified in the additional 6196 transcripts sequenced, the removal of data from a breast tumor with an abnormally high passenger mutation rate, the use of an experimental rather than statistical definition of *CAN*-genes, and additional evaluation of mutations in samples that had undergone whole-genome amplification (*7*).

It is reasonable to assume that genes that are mutated more frequently than predicted by chance are more likely to be drivers. In this study, we used a more sophisticated version of a metric, called the cancer mutation prevalence (CaMP) score, to rank genes by the number and nature of the mutations observed (table S4, A and B). To assess the likelihood that each of these genes is mutated at a frequency higher than the passenger mutation rate, we devised a method based on Empirical Bayes simulations (*7*). Though the likelihoods depend on the passenger rates (table S4, A and B), the rankings of the genes by CaMP scores are similar regardless of the assumed passenger mutation rates (rank correlations > 0.9). CaMP scores thereby provide priorities for future studies that are independent of many of the assumptions required to calculate passenger probabilities.

To determine the mutation prevalence of a subset of *CAN*-genes with more precision, we analyzed 40 *CAN*-genes in a separate cohort of 96 patients with colorectal cancers (*7*). The genes chosen were in biological pathways of interest to our groups and included those ranked 1st to 119th by CaMP scores. Colorectal cancers, rather than breast tumors, were chosen because more purified tumor tissues of this type were available. Twenty-five of the 40 genes (62%) were found to be mutated in at least one of the 96 cancers and, as predicted from our data and simulations, most were mutated in 5% or less of the cancers (table S5). The remaining 15 *CAN*-genes were not mutated in any of the additional 96 cancers studied, but this finding is still compatible with these genes being mutated in a low but significant fraction of tumors; the evaluation of more colorectal tumors than the 131 included in our study would be necessary to exclude this possibility.

Additional analyses of mutated genes. Mutation frequency is not the only type of information that can help determine whether a mutated gene is worthy of further evaluation. The analyses of the predicted effects on protein function can add independent evidence helpful for prioritization of specific genes and mutations for future research. For example, mutations producing stop codons, out-of-frame insertions or deletions, or splice site abnormalities are very likely to interfere with the normal function of the gene product (tables S3 and S4). To evaluate missense changes, we used two sequence-based methods for evaluating the probability that a specific alteration would have a deleterious effect on protein function: Sorting Intolerant from Tolerant (SIFT) and LogR.E-values based on Pfam domains (*7*). These probabilities are listed for each evaluable mutation identified in our study in table S3. For each *CAN*-gene, the number of missense mutations that were predicted to disrupt function in a statistically significant manner is included in table S4.

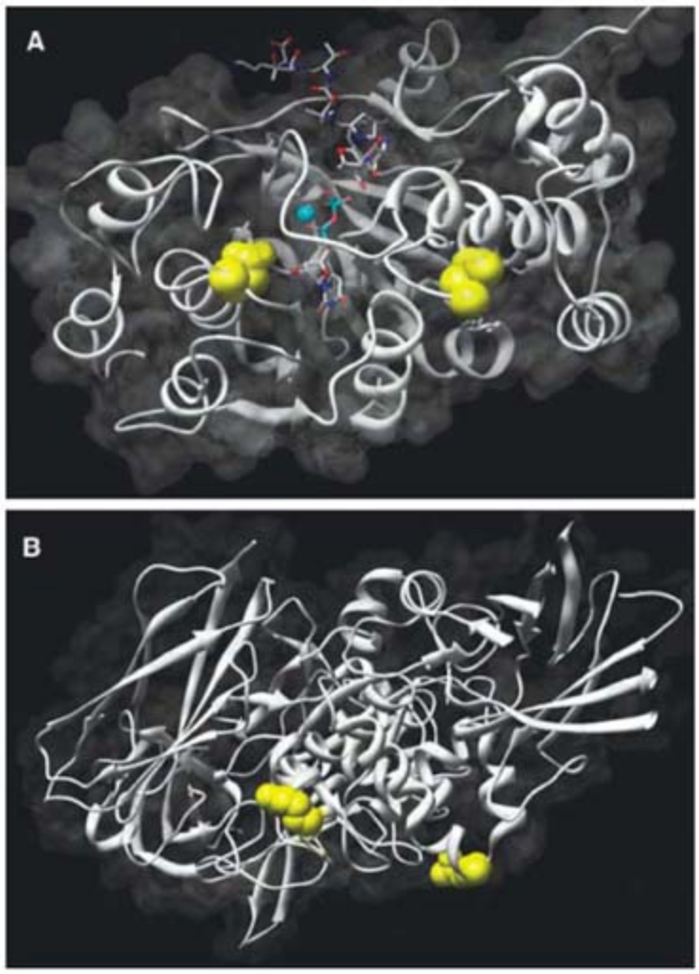

Fig. 1. Clustering of somatic mutations in protein structures. Individual somatic mutations were mapped onto structural homology models on the basis of known crystal structure information. Homology models were built with MODPIPE (*33*) and graphics were created with UCSF Chimera software (*34*). Yellow spheres indicate mutated residues. (**A**) Two somatic mutations in the glycosylation enzyme *GALNT5* occur in residues on different sides of the enzyme active site. Stick models indicate enzyme substrates. (**B**) Three somatic mutations in the transglutaminase *TGM3* located at nearby surface regions of the protein (two mutations are present at the same residue on the right-hand side).

Predictions about the functional effects of mutations can also be made at the structural level. We generated structural models for 622 of the RefSeq gene mutations from x-ray crystallography or nuclear magnetic resonance spectroscopy of their encoded or related proteins (*12*, *13*). Some of the models were intriguing in that they showed clustering of mutations around active sites of proteins or near an interface residue (examples in Fig. 1). We also used LS-SNP software (*14*) to predict the likelihood that each mutation would destabilize the protein, interfere with the formation of a domain-domain interface, or have an effect on protein-ligand binding (table S3, summarized for *CAN*-genes in table S4).

Finally, we identified a number of mutations that occurred at locations identical to those of genes involved in hereditary human diseases or that clustered at adjacent locations in the cancers analyzed. Such alterations are likely to have functional effects on these proteins. These included the R360W mutation (substitution of arginine 360 with tryptophan) in the *RET* tyrosine kinase, corresponding to an identical loss-of-function germline change in Hirschsprung disease (*15*). Likewise, the R1624W mutation in the *PKHD1* gene in colorectal cancer is identical to that observed in polycystic kidney disease, a syndrome that has neoplastic features (*16*). The T745M mutation (substitution of threonine 745 with methionine) in the cell adhesion gene *CRB1* gene is identical to one that has been shown to be a cause of retinitis pigmentosa (*17*). In addition to these examples, we identified 126 mutations in 39 proteins that occurred within a distance of 10 amino acids from one another. In particular, mutations in at least two independent tumors occurred in the *DTNB*, *EDD1*, *GNAS*, and *TGM3* genes at exactly the same residue, implicating that region as vital to the protein's potential tumorigenic function.

Analysis of mutated pathways. It is becoming increasingly clear that pathways rather than individual genes govern the course of tumorigenesis (*1*). Mutations in any of several genes of a single pathway can thereby cause equivalent increases in net cell proliferation. Accordingly, we devised a method to determine whether the genes within specific pathways were mutated more often than predicted by chance. The resultant "pathway CaMP" score incorporated the total number of mutations from all genes within each group, the number of different genes mutated, the combined sizes of the genes in each group, and the total number of tumors examined (table S6) (*7*).

Using this metric, we analyzed a highly curated database (Metacore, GeneGo, Inc.) that includes human protein-protein interactions, signal transduction and metabolic pathways, and a variety of cellular functions and processes. By including the number of mutated genes in addition to the total number of mutations as parameters, we excluded pathways that simply contained one gene that was mutated at high frequency (e.g., pathways containing only *TP53* mutations). There were 108 pathways that were found to be preferentially mutated in breast tumors. Many of the pathways involved phosphatidylinositol 3-kinase (PI3K) signaling (Fig. 2 and table S6B). Mutations in *PIK3CA* are frequent in multiple tumor types, including breast cancers (*18*–*21*). In this study, we identified mutations not only in *PIK3CA*, but also previously unreported mutations in *GAB1*, *IKBKB*, *IRS4*, *NFKB1*, *NFKBIA*, *NFKBIE*, *PIK3R1*, *PIK3R4*, and *RPS6KA3*, implicating both the PI3K pathway in general and nuclear factor κB (NF-κB) signaling in particular in breast tumorigenesis. Within the 38 colorectal cancer pathways that appeared to be mutated in a statistically significant manner, there were also many that centered on PI3K (table S6A). The pathway components mutated in colorectal cancers differed from those in breast, with mutations found in *IRS2*, *IRS4*, *PIK3R5*, *PRKCZ*, *PTEN*, *RHEB*, and *RPS6KB1* in addition to *PIK3CA*. Additional pathways altered in colorectal cancer were related to cell adhesion, the cytoskeleton, and the extracellular matrix (table S6A), supporting the idea that interactions between the cancer cell and the extracellular environment are important steps in the neoplastic process.

Finally, there were nine examples of mutated genes whose protein products were predicted to interact with other mutated genes more often than predicted by chance. The average number of mutant gene products with which these nine mutant genes interacted was 25 (table S6). These results illustrate the potential utility of pathway-based analyses and highlight a variety of different gene groups and pathways that can help focus further investigations on these tumor types.

The genomic landscapes of colorectal and breast cancers. The colorectal and breast cancers analyzed in the Discovery Screen contained a median of 76 and 84 nonsilent mutations in RefSeq genes, respectively (table S2). The number of mutations per tumor was similar among colorectal tumors (ranging from 49 to 111) but was more variable in breast cancers (varying from 38 to 193). The number of mutated *CAN*-genes per tumor averaged 15 and 14 in colorectal and breast cancers, respectively.

The "landscapes" of typical colorectal and breast cancer genomes are depicted in Fig. 3. In these landscapes, every RefSeq gene is represented by a point on a two-dimensional map corresponding to its chromosomal position, and all mutated genes in that tumor are indicated by a dot. The relief feature of the map is provided by the *CAN*-genes with the 60 highest CaMP scores (table S4). Just as topographical maps contain geological features of varying elevations, the cancer genome landscape consists of relief

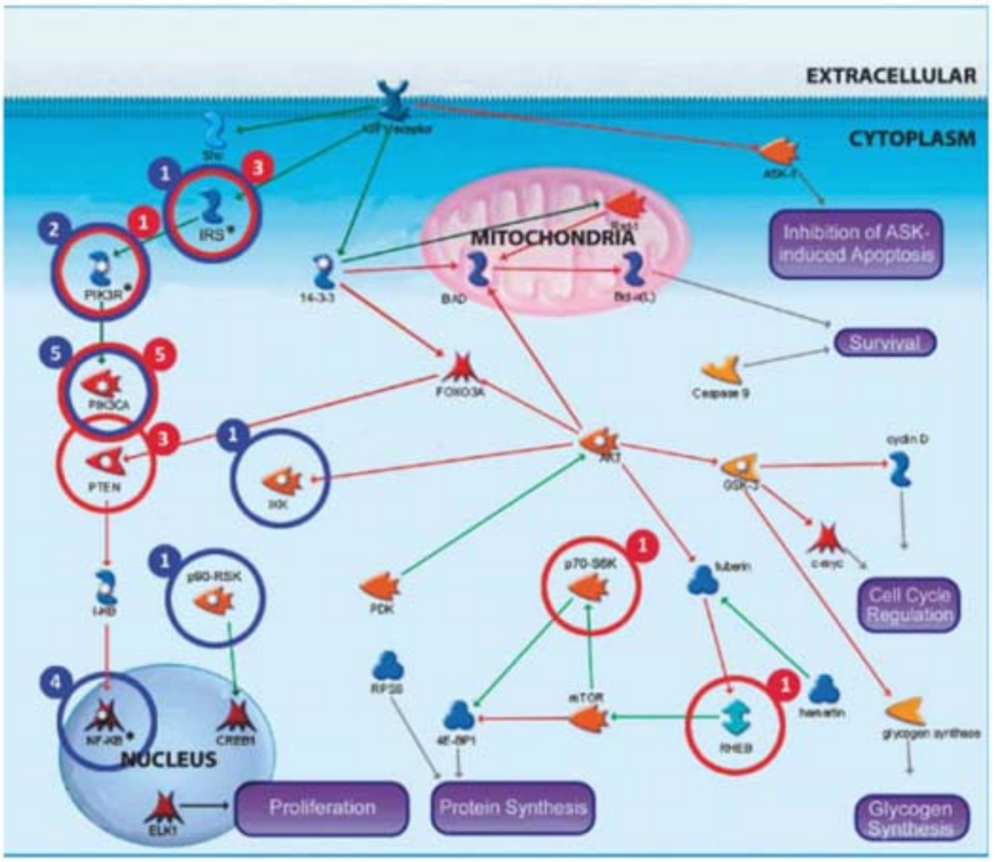

Fig. 2. PI3K pathway mutations in breast and colorectal cancers. The identities and relationships of genes that function in PI3K signaling are indicated. Circled genes have somatic mutations in colorectal (red) and breast (blue) cancers. The number of tumors with somatic mutations in each mutated protein is indicated by the number adjacent to the circle. Asterisks indicate proteins with mutated isoforms that may play similar roles in the cell. These include insulin receptor substrates IRS2 and IRS4; phosphatidylinositol 3-kinase regulatory subunits PIK3R1, PIK3R4, and PIK3R5; and NF-κB regulators NFKB1, NFKBIA, and NFKBIE.

features (mutated genes) with heterogeneous heights (determined by CaMP scores). There are a few "mountains" representing individual *CAN*-genes mutated at high frequency. However, the landscapes contain a much larger number of "hills" representing the *CAN*-genes that are mutated at relatively low frequency. It is notable that this general genomic landscape (few gene mountains and many gene hills) is a common feature of both breast and colorectal tumors.

Discussion. The results reported here add to those published previously (*5*) in several important ways. First, we report the sequences of an additional 5168 genes in 22 tumors. These new data provide a much more complete picture of the cancer genome, allowing us to formulate landscapes of breast and colorectal tumors (Fig. 3). We predict that the key features of this landscape—a few gene mountains interspersed with many gene hills—will prove to be a general feature of most solid tumors. Second, we present data on noncoding and synonymous mutations in addition to nonsynonymous mutations. As well as providing information useful for estimating the passenger rate, the data in table S2 show that passenger rates vary considerably from tumor to tumor, undoubtedly determined by their intrinsic mutability and the number of generations and bottlenecks through which they have evolved. Third, we present more sophisticated methods for identifying and classifying genes with more mutations than predicted by the passenger rate (table S4). Fourth, we present a variety of tools based on gene products' sequence and structure, as well as their inclusion in certain pathways, that can help identify mutated genes that are most deserving of further attention (Figs. 1 and 2 and tables S3, S4, and S6). These tools can be used to prioritize the research that follows cancer genome-sequencing efforts.

In terms of such research, it is important to note that sequence data can inform other, independent approaches to the study of cancer genes. For example, chromodomain helicase DNA binding domain 5 (*CHD5*) was recently proposed to be a tumor suppressor on the basis of its functional properties and copy-number alterations (*22*). We identified somatic mutations in this gene in breast tumors; the combined data strongly support a role for this gene in tumorigenesis. Similarly, the NF-κB pathway member *IKBKE* was recently suggested to be a breast cancer oncogene on the basis of functional and expression studies (*23*). We found somatic mutations in several additional components of this signaling pathway (Fig. 2), reinforcing its importance in breast cancers. The transglutaminase (TGM) enzymes have recently been implicated in invasion and metastasis (*24*), and we identified multiple somatic mutations in *TGM3* in colorectal cancers (Fig. 1). Additionally, a high-throughput retroviral insertional mutagenesis screen in mouse mammary tumor virus (MMTV)-induced mammary tumors in mice identified 33 common insertion sites as potential oncogenes (*25*); we found 7 of these 33 genes to be mutated in breast cancers. Given the entirely independent nature of these screens (insertional mutagenesis in mouse versus mutational analysis of human genes), the overlap of these results is remarkable.

Historically, the focus of cancer research has been on gene mountains, in part because they were the only alterations identifiable with available technologies. The ability to analyze the sequence of virtually all protein-encoding genes in cancers has shown that the vast majority of mutations in cancers, including those that are most likely to be drivers, do not occur in such mountains and emphasize the heterogeneity and complexity of human neoplasia. This new view of cancer is consistent with the idea that a large number of mutations, each associated with a small fitness advantage, drive tumor progression (*26*). But is it possible to make sense out of this complexity? When all the mutations that occur in different tumors are summed, the number of potential driver genes is large. But this is likely to actually reflect changes in a much more limited number of pathways, numbering no more than 20 (*1*). This interpretation is consistent with virtually all screens in model organisms, which have generally shown that the same phenotype can arise from alterations in any of several genes. Other recent studies lend support to this interpretation. For example, sequencing studies of the kinome in large numbers of tumors have shown that specific kinases are sometimes mutated in a small fraction of tumors of a given type (*4*, *10*, *27*–*29*). We cannot be certain that the bulk of the low-frequency mutations observed in our study are not passengers. However, in the kinome studies, the position of mutations within the activation loop and the demonstrated effects of the target residues on kinase function unambiguously implicate many of these rare mutations as drivers. Similarly, recent analyses of myelomas suggest that there are multiple genes, each mutated in a small proportion of tumors, that can alter the same signal transduction pathway (*30*, *31*). Furthermore, some of the low-frequency mutations observed in our study, such as activating mutations in the guanine nucleotide binding protein GNAS and a homozygous nonsense mutation in BRCA1-associated protein (BAP1), are likely to be functional (table S3). These examples, in addition to those in table S6, bolster the argument that infrequent mutations can be drivers and that they function through pathways that are already known.

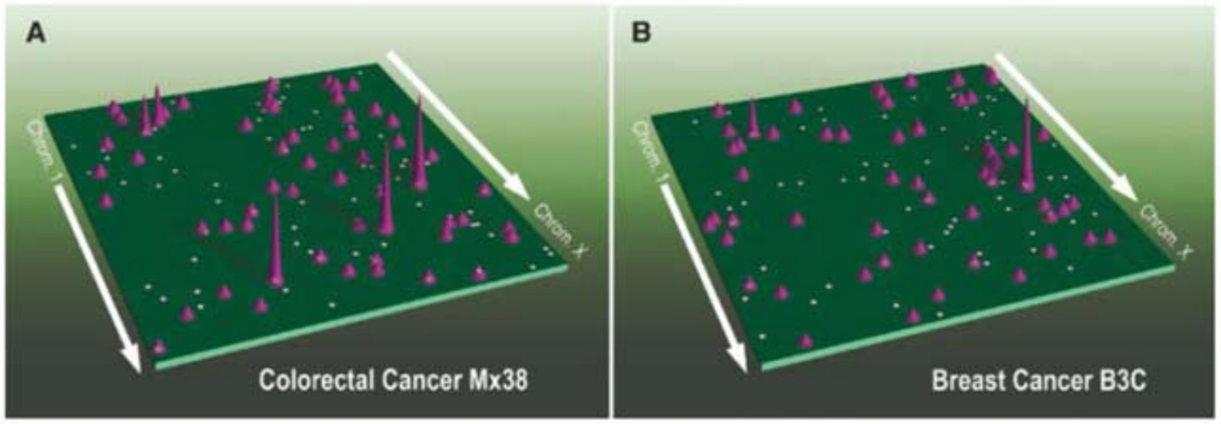

Fig. 3. Cancer genome landscapes. Nonsilent somatic mutations are plotted in two-dimensional space representing chromosomal positions of RefSeq genes. The telomere of the short arm of chromosome 1 is represented in the rear left corner of the green plane and ascending chromosomal positions continue in the direction of the arrow. Chromosomal positions that follow the front edge of the plane are continued at the back edge of the plane of the adjacent row, and chromosomes are appended end to end. Peaks indicate the 60 highest-ranking *CAN*-genes for each tumor type, with peak heights reflecting CaMP scores (*7*). The dots represent genes that were somatically mutated in the individual colorectal (Mx38) (**A**) or breast tumor (B3C) (**B**) displayed. The dots corresponding to mutated genes that coincided with hills or mountains are black with white rims; the remaining dots are white with red rims. The mountain on the right of both landscapes represents *TP53* (chromosome 17), and the other mountain shared by both breast and colorectal cancers is *PIK3CA* (upper left, chromosome 3).

Regardless of whether this pathway-centric interpretation is correct, it is clear that the "easy" part of future cancer genome research will be the identification of genetic alterations. The vast majority of subtle mutations in individual patient's tumors can now be identified with existing technology (Fig. 3), making personal cancer genomics a reality. Though understanding the precise role of these genetic alterations in tumorigenesis will be more challenging, opportunities for exploiting such personal genomic data on cancers are already apparent. For example, many of the genes altered in breast cancers appear to affect the NF-κB pathway (table S6), suggesting that drugs targeting this pathway could be efficacious in breast cancers with such mutations (*30*, *31*). Furthermore, our data indicate that individual breast and colorectal cancers each contain ~80 amino acid–altering mutations that are absent in all normal cells, providing a wealth of opportunities for personalized immunotherapy. Finally, any mutation identified in an individual cancer, whether driver or passenger, can be used as an exquisitely specific biomarker to guide patient management (*32*).

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Supporting Online Material

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Materials and Methods
Statistical Analysis Package
Fig. S1
Tables S1 to S6
References

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Orbital Reconstruction and Covalent Bonding at an Oxide Interface

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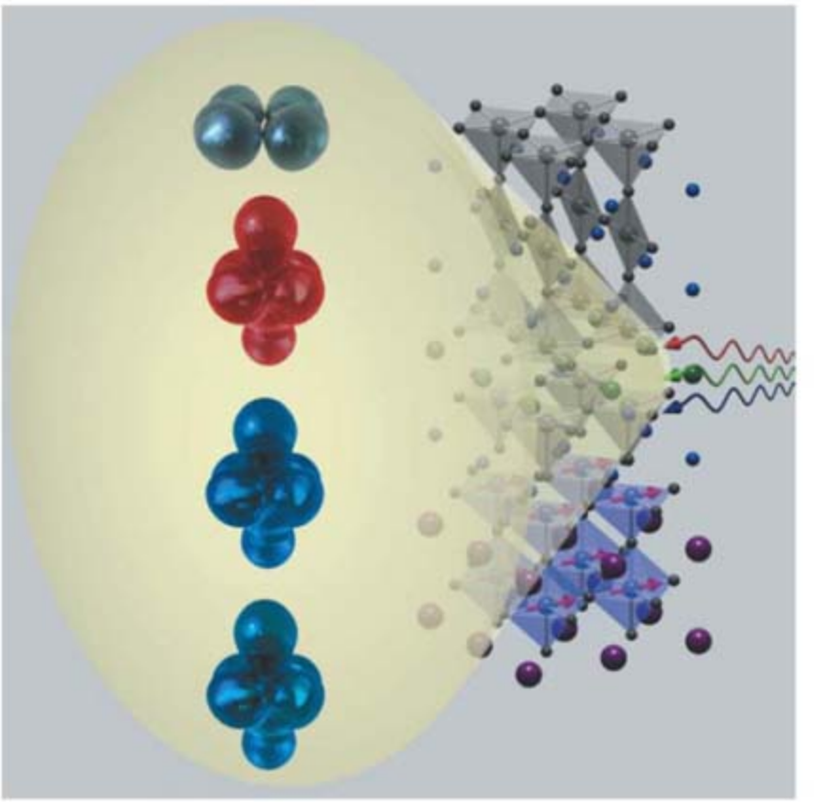
AUTHORS' SUMMARY

Modern microelectronics relies to a large degree on the properties of interfaces between two different semiconductors. For example, a transistor is commonly formed from the electronic interactions at such an interface, and its electrical conductivity can be controlled very effectively by application of an external voltage to its gate electrode. Optimizing the operation of transistors and other microelectronic devices requires knowledge of the electronic states near semiconductor interfaces, and many details have been revealed over the past half-century. Key to recent developments has been the ability to create high-quality atomically abrupt interfaces between different materials, including complex oxides with intricate or large unit cells. In bulk, these oxides show rich and varied electronic and magnetic properties produced by strong interactions among the electrons (*1*). Combining these materials along an interface can produce new quantum states and the opportunity to uncover unexpected phenomena. We outline steps toward visualizing and resolving the detailed behavior of electrons in specific orbitals at the interface between two complex materials. We studied two materials exhibiting properties not known in ordinary semiconductors: a ferromagnetic manganese oxide and a superconducting copper oxide. The ability to manipulate electrons and their behavior at interfaces may open a path toward a new generation of electronic devices.

The first step along this path is the preparation of chemically pure, atomically sharp interfaces. A sharp interface is needed both to accurately study and to constrain electronic interactions to the interface. We used pulsed laser deposition, in which a laser vaporizes bulk samples in a vacuum containing some background oxygen. A series of thin, uniform oxide layers is sequentially deposited on a single crystal forming a superlattice with sharp interfaces. Study of similar structures during the past few years has led to demonstrations of high-mobility electron systems (*2*) and transistor effects (*3*) at oxide interfaces.

Understanding the electronic mechanisms responsible for these effects requires the ability to study the behavior of electrons at the interfaces, which are typically buried several nanometers below the surface, without interference from bulk electrons in each layer of the superlattice. To meet this goal, we rely on the properties of x-rays with tunable energy and polarization emitted from a synchrotron, which penetrate deeply into most materials. The x-ray photon energy was tuned to zoom in on copper and manganese atoms right at the interface, and the absorption of x-rays with polarization parallel and perpendicular to the interface was used to extract information about the shape of the valence-electron clouds (i.e., "orbitals") around these atoms (see the figure).

In analogy to atoms in free space, the electrons in metal oxides have the choice of several types of energetically nearly equivalent (or degenerate) electronic orbitals. The specific way in which this freedom is broken strongly influences the interactions between the electrons and hence the physical properties of bulk transition metal oxides. For instance, alternating occupation of different orbitals on neighboring lattice sites favors ferromagnetism, whereas uniform occupation of orbitals on all lattice sites tends to generate antiferromagnetism (*1*). Early investigations with x-rays had established that the arrangement of valence-electron orbitals on copper atoms in bulk copper-oxide superconductors is particularly robust, so that there is essentially no degeneracy, and virtually all theories of high-temperature superconductivity are now based on this orbital pattern. Our data indicate that electrons at the interface occupy a combination of orbitals that differs drastically from that of the bulk. With the aid of numerical calculations on small atomic clusters, we trace this "orbital reconstruction" at the interface to the formation of strong covalent bonds between copper and manganese atoms across the interface.



Schematic showing the interface between two metal oxide compounds being illuminated by x-rays from a synchrotron, yielding detailed information about the shape, and thus occupation and degeneracy, of electronic orbitals near the interface.

Being able to determine these characteristics known only in the interface between metal oxides should allow synthesis of materials in which the bonding across oxide interfaces can be manipulated in a predictable fashion. These methods then will offer a tremendous opportunity to create dense two-dimensional electron systems with controlled interactions. It is conceivable that such a system will exhibit properties qualitatively beyond those attainable in semiconductor heterostructures and that engineers will be able to exploit these properties in innovative electronic devices.

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FULL-LENGTH ARTICLE

Orbital reconstructions and covalent bonding must be considered as important factors in the rational design of oxide heterostructures with engineered physical properties. We have investigated the interface between high-temperature superconducting (Y,Ca)$Ba_2Cu_3O_7$ and metallic $La_{0.67}Ca_{0.33}MnO_3$ by resonant x-ray spectroscopy. A charge of about −0.2 electron is transferred from Mn to Cu ions across the interface and induces a major reconstruction of the orbital occupation and orbital symmetry in the interfacial CuO_2 layers. In particular, the Cu $d_{3z^2-r^2}$ orbital, which is fully occupied and electronically inactive in the bulk, is partially occupied at the interface. Supported by exact-diagonalization calculations, these data indicate the formation of a strong chemical bond between Cu and Mn atoms across the interface. Orbital reconstructions and associated covalent bonding are thus important factors in determining the physical properties of oxide heterostructures.

In semiconductor heterostructures, high-mobility electron systems with tunable density have led to prominent advances in science and technology over the past decades. Such systems have recently been replicated in heterostructures of complex transition metal oxides (*1*), leading to the observation of transistor effects (*2*) and the quantum Hall effect (*3*). Because transition metal oxides exhibit a notably rich phase behavior in the bulk (*4*), these developments have raised expectations that quantum states with properties and functionalities qualitatively beyond those attainable in semiconductors can be generated at oxide interfaces.

The large variety of phases (often with radically different physical properties) in transition metal oxides is due to the delicate sensitivity of the charge transfer and magnetic interaction between metal ions to the occupation of *d* orbitals (*5*). Which linear combination of the five possible *d* orbitals is occupied on a given transition metal site depends, in turn, on parameters such as electron density, ligand positions, magnetic order, and chemical bonding, which are generally different at the interface than in the bulk. Despite its pivotal role in determining the phase behavior and physical properties of oxides, almost no experimental information is available about the occupation of orbitals at oxide interfaces, and theoretical work (*6*–*11*) has thus far hardly addressed this issue. We report the results of soft x-ray absorption spectroscopy (XAS) and soft x-ray linear dichroism (XLD) experiments on heterostructures of copper and manganese oxides tailored to probe the electronic structure and orbital occupation at the interface. The cuprate-manganate interface is well suited as a model system for this purpose, because nearly strain-free, atomically sharp heterostructures can be synthesized (*12*–*14*) and because the electronic properties of both materials have been studied extensively in the bulk.

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Probing heterostructure interfaces. The experiments were performed at the 4-ID-C beamline at the Advanced Photon Source on epitaxial trilayers and superlattices of the high-temperature superconductor (Y,Ca)$Ba_2Cu_3O_7$ (YBCO) in *c*-axis orientation, combined with ferromagnetic metallic $La_{1-x}Ca_xMnO_3$ (LCMO) at a doping level $x = 1/3$. The quality of these multilayer structures was checked by a variety of characterization methods (*15*). In order to discriminate the electronic structure at the interface from surface and bulk contributions, we have performed a systematic series of experiments on heterostructures with different capping layers, taking advantage of the element specificity and shallow probing depth of resonant XAS and XLD in the total electron yield (TEY) mode (Fig. 1A). For instance, the occupation of Cu *d* orbitals on the YBCO side of the interface was studied on heterostructures with LCMO capping layers, so that no surface Cu is present. If the photon energy is tuned to the Cu L absorption edge, the capping layer does not influence the detected signal apart from an overall attenuation factor. As a result of the low electron escape depth (a few nanometers), the TEY signal is dominated by the CuO_2 layers immediately adjacent to the first interface; contributions from deeper layers are exponentially reduced. The interface sensitivity is further enhanced with the use of a low angle of incidence for the x-ray beam (11.2°). The converse procedure was used to probe the electronic structure of MnO_2 layers on the LCMO side of the interface. Control experiments in the bulk-sensitive fluorescence-yield (FY) mode were simultaneously carried out in both cases.

Spectroscopy at the interface. Figure 2 shows normalized absorption spectra near the Cu L_3 edge in bulk- and interface-sensitive modes. The bulk-sensitive FY data are in excellent agreement with previous XAS data at the Cu L edge of nearly optimally doped YBCO (*16*). The main narrow absorption peak around 931 eV corresponds to the intra-ionic transition $2p^63d^9 \rightarrow 2p^53d^{10}$. The shoulder on the right-hand side of the peak is attributed to the intersite transition $2p^63d^9\underline{L} \rightarrow 2p^53d^{10}\underline{L}$, where $\underline{L}$ denotes a hole on the oxygen ligand. The line shape of the main absorption peak is a signature of the "Zhang-Rice singlet" (*17*), a bound state of charge carriers on oxygen and copper sites that keeps the Cu plane site in the nominal valence state 2+ as the hole density in the CuO_2 sheets is tuned by doping. The polarization dependence of the FY signal also contains important information about the electronic structure near the Fermi level of YBCO. In particular, the absorption for photon polarization parallel to the CuO_2 sheets greatly exceeds that for polarization along the *c* axis. This implies that holes in the conduction band of YBCO predominantly occupy the planar Cu $d_{x^2-y^2}$ orbital, which hybridizes strongly with oxygen *p* orbitals in the CuO_2 layers. Similar observations have been made in all other high-temperature superconductors investigated thus far, and together they have become one of the basic tenets of our current understanding of this class of materials (*16*, *18*).

Evidence for orbital reconstruction and charge transfer. The interface-sensitive data shown in Fig. 2 are very different. One first notices that

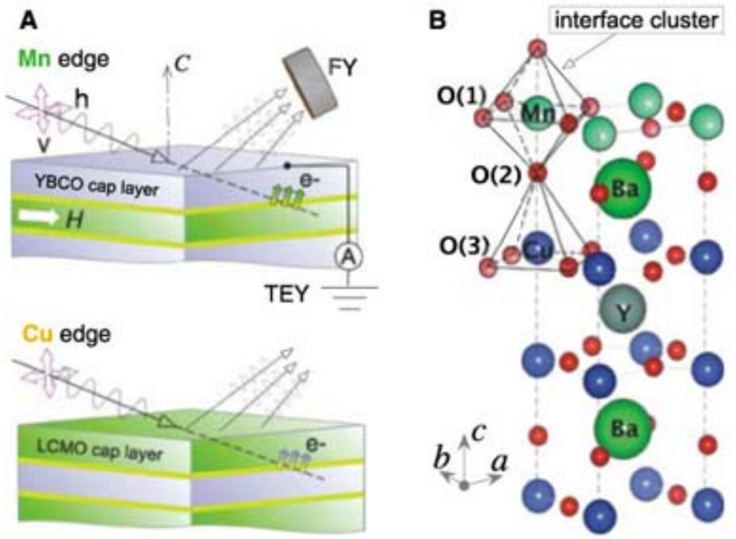

Fig. 1. (**A**) Schematic of the experimental setup used to obtain the XAS and XLD data in TEY and FY modes. Data sensitive to interfacial Cu (Mn) atoms were taken in TEY mode with photon energies near the Cu (Mn) L absorption edge, on samples with LCMO (YBCO) capping layers. To obtain a sizable dichroism, we tilted the film plane with respect to the photon beam propagation direction. *C* indicates the *c*-axis of the film; *H* is the applied magnetic field; *h* and *v* denote the linear polarization state of the incident x-ray. (**B**) Atomic positions near the LCMO-YBCO interface (*14*, *29*). The $MnCuO_{10}$ cluster used for the exact-diagonalization calculations is highlighted.

the interfacial absorption peak is shifted to lower energy with respect to the bulk by ~0.4 eV and that the high-energy shoulder is no longer present. The shift of the peak is evidence of a change in valence state of Cu ions near the interface. This indicates that charge is transferred across the interface and that a charged double layer is formed, as generally expected for heterostructures of materials with different work functions. In agreement with specific predictions for the system at hand (*11*), the charge-transfer direction is such that the hole density in YBCO is reduced at the interface. Because of the strong influence of the core hole created by absorbing the photon, the relationship between the x-ray absorption edge and the Cu valence is not straightforward, but a comparison to XAS spectra of reference materials containing Cu^{1+} and Cu^{2+} ions [for a review, see (*19*)] yields a rough estimate of $0.2e$ (where e in the charge on the electron) per copper ion for the charge-transfer amplitude. At first sight, this seems to correspond with the line shape of the interfacial absorption peak, which bears a strong resemblance to XAS data in undoped YBCO (*16*). Notably, however, numerous XAS experiments on YBCO and other bulk hole-doped high-temperature superconductors have shown that the position of the Cu L-absorption peak is independent of doping. This has been attributed to the Zhang-Rice singlet state and, consequently, the doped holes have predominantly oxygen character (*17*). The observed shift of the L_3 absorption peak in our interface-sensitive experiment thus cannot be attributed to a readjustment of the hole density alone and indicates an extreme modification of the electronic structure of the CuO_2 layer adjacent to the interface.

In order to uncover the origin of the unexpected shift of the absorption peak and to obtain further information about the electronic states at the interface, we have varied the photon polarization in the interface-sensitive detection mode (Fig. 2). In marked contrast to the bulk-sensitive data, the strengths of the absorption signals for polarization perpendicular and parallel to the layers are almost equal. This is a manifestation of an "orbital reconstruction." Whereas the holes are constrained to the Cu $d_{x^2-y^2}$ orbital in the bulk, at least some of them occupy the $d_{3z^2-r^2}$ orbitals at the interface. The distribution of holes over the two Cu orbitals cannot be precisely determined, because the XLD experiment probes not only the CuO_2 layer directly at the interface but also the deeper layers (albeit with exponentially reduced sensitivity). However, the nearly isotropic cross section shown in Fig. 2 implies that the hole content of the Cu $d_{3z^2-r^2}$ orbital is at least equal to that of the $d_{x^2-y^2}$ orbital. We repeated the measurement at several temperatures (from 300 to 30 K) and confirmed that the peak position and polarization dependence do not depend on temperature. Similar observations were also made on heterostructures in which the doping level of YBCO was raised into the overdoped regime by Ca substitution. The orbital reconstruction and the charge transfer are hence general, robust characteristics of the YBCO-LCMO interface.

Before discussing possible mechanisms and potential implications of the orbital reconstruction, we briefly discuss XAS spectra near the Mn L_2 and L_3 absorption edges taken in bulk- and interface-sensitive modes (Fig. 3). The bulk-sensitive data are again in good agreement with corresponding data in the literature. The spectra are much broader than those taken near the Cu L edge, because all of the five Mn d orbitals are partially occupied, giving rise to a complicated multiplet splitting of the absorption peak. The peak intensity is independent of photon polarization within the experimental error. This finding has been taken as evidence of an orbitally disordered state with equal occupation of Mn $d_{x^2-y^2}$ and $d_{3z^2-r^2}$ orbitals in bulk metallic LCMO. In the interface-sensitive detection mode, neither the peak position nor its polarization dependence are noticeably different from the bulk data. This does not imply, however, that the Mn ions maintain their bulk charge density and electronic structure at the interface. Indeed, as a result of charge conservation, one generally expects a shift in Mn valence matching that of the interfacial Cu ions (Fig. 2), but because of the strong multiplet broadening of the Mn peak, such a shift is much harder to recognize than in the case of Cu (*20*). Based on the data of Fig. 3, one can set an upper bound of 0.4 eV on the difference between the positions of Mn L absorption edges in bulk- and interface-sensitive detection modes. Because a valence change from Mn^{3+} to Mn^{4+} results in a shift of the L edge of ~1.5 eV, this translates into an upper bound of ~$0.3e$ per Mn atom on the amplitude of the charge transfer across the interface, which is consistent with the estimated amplitude of ~$0.2e$ based on the Cu XAS spectra discussed above. Likewise, because the polarization dependence of the intensity at the Mn L edge is influenced to a large extent by the completely unoccupied minority t_{2g} and e_g orbitals, it is difficult to see a rearrangement of the majority $d_{x^2-y^2}$ and $d_{3z^2-r^2}$ orbitals comparable to that observed on Cu.

Mechanism of orbital reconstruction. Our data therefore imply that the interfacial Cu $d_{3z^2-r^2}$ orbitals, which are fully occupied in bulk YBCO, are partially populated by holes at the interface. In principle, two distinct physical mechanisms could lead to such an orbital reconstruction. First, it is possible that the different crystal-field environment of Cu ions at the interface could raise the energy of the $d_{3z^2-r^2}$ orbital above that of the $d_{x^2-y^2}$ orbital. Because the ligand positions at the interface are not precisely known, this scenario cannot be firmly ruled out, but it is highly unlikely because of the large energy difference between Cu $d_{3z^2-r^2}$– and $d_{x^2-y^2}$–derived bands in bulk YBCO. A reversal of this hierarchy would require a substantially shorter distance between the copper and apical oxygen O(2) ions as compared with the in-plane Cu-O bond length, which is unrealistic. Furthermore, the major difference between the bulk and interface crystal-field environments is the substitution of Cu-chain ions (with valence close to 2+ in bulk YBCO) by Mn ions (with valence ~3.3+ in bulk LCMO). The higher ligand charge should lower the energy of the $d_{3z^2-r^2}$ orbital and further increase the energy difference with the $d_{x^2-y^2}$ level. A major rearrangement of the orbital level scheme due to

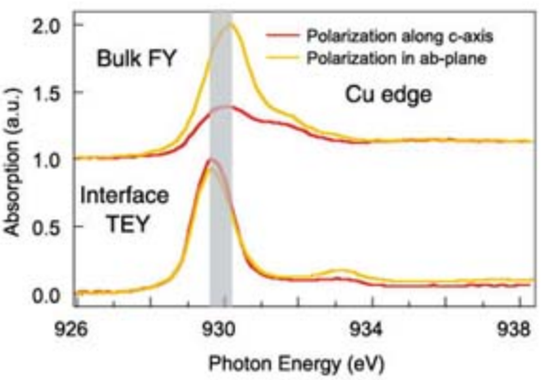

Fig. 2. Normalized x-ray absorption spectra at the Cu L_3 absorption edge, taken in bulk-sensitive (FY, top panel) and interface-sensitive (TEY, bottom panel) detection modes with varying photon polarization as indicated in the legend. a.u., arbitrary units.

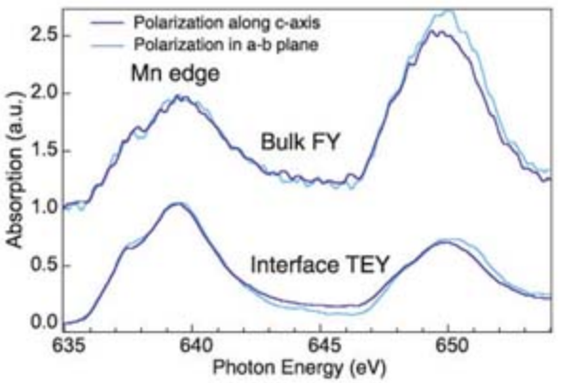

Fig. 3. Normalized x-ray absorption spectra at the Mn L_2 and L_3 absorption edges, taken in bulk-sensitive (FY, top panel) and interface-sensitive (TEY, bottom panel) detection modes with varying photon polarization as indicated in the legend. The line shape of the FY spectra is distorted by self-absorption effects.

readjustments of ligand positions is therefore implausible. The second scenario is based on the observation that the Cu $d_{3z^2-r^2}$ orbital points directly toward the interface and can hybridize effectively with the Mn $d_{3z^2-r^2}$ orbital via the apical oxygen ion [O(2) in Fig. 1B], generating a covalent chemical bond bridging the interface. In this scenario, covalency results in the formation of extended "molecular orbitals" consisting of atomic Cu and Mn $d_{3z^2-r^2}$ orbitals with an admixture of the p_z orbitals on the apical oxygen (insets in Fig. 4).

Covalent bonding at the interface. To check whether the covalent-bonding scenario is viable, we performed exact-diagonalization calculations of the $MnCuO_{10}$ cluster shown in Fig. 1B, including a single hole and the full set of Cu *d* orbitals and interaction parameters described in the literature (*15*, *21*). Because the Mn $d_{x^2-y^2}$ orbital does not hybridize with Cu, the Mn ion is represented by a single $d_{3z^2-r^2}$ orbital with a classical Hund's rule coupling to the core t_{2g} spins. In order to simulate the difference in YBCO and LCMO work functions, we tuned the on-site energy of the hole on Mn. Figure 4 shows the hole density in the Cu *d* shell (measured from the full-shell configuration $3d^{10}$) as a function of this parameter. For large values, the hole resides completely on the Cu ion, and the ionic valencies are close to their bulk values (i.e., Cu is in the formal valence state 2+, and the Mn valence is 3+). The formal Cu^{2+} valence state, realized at high Mn hole on-site energy, corresponds to ~0.76 holes in the Cu *d* shell, which reside in the Cu $d_{x^2-y^2}$ orbital. The remaining hole density is in the in-plane oxygen *p* orbitals [O(3) in Fig. 1B], which hybridize strongly with Cu $d_{x^2-y^2}$. The plot also shows that the charge transfer across the interface leads to a major rearrangement of the electron distribution in the Cu e_g orbital manifold. Indeed, when the transfer is complete, the Cu $d_{x^2-y^2}$ orbital is completely full, and holes partially occupy the $d_{3z^2-r^2}$ orbital. This indicates that the Mn and Cu $d_{3z^2-r^2}$ orbitals are indeed strongly hybridized and that molecular orbitals are formed. The charge-transfer transition occurs when the antibonding molecular orbital crosses the Cu $d_{x^2-y^2}$ level, as shown in the insets. The change in hole density on Cu induced by the charge-transfer transition is another signature of the formation of a covalent bond: Figure 4 shows that only about half of the hole charge ends up on Cu; the remaining hole charge is distributed over the Mn- and O(2)-derived components of the molecular orbital.

The cluster calculation demonstrates that the formation of a strong covalent bond between Cu and Mn ions at the interface is indeed realistic. Partial occupancy of the corresponding antibonding molecular orbital qualitatively explains the modification of the XLD spectra at the interface. The filling factor of this orbital is expected to match that of the Mn $d_{3z^2-r^2}$ orbital, which is about one-third in bulk LCMO. According to the calculation, a substantial fraction of the charge density in the hybrid orbital resides on Cu, which is in good agreement with the experimentally observed shift of the Cu valence. Because holes in this orbital are not subject to Zhang-Rice singlet formation, the shift of the Cu XAS peak at the interface is also explained. Also, the Cu-Mn orbital hybridization naturally results in a strong antiferromagnetic exchange coupling (*15*), as recently observed at LCMO-YBCO interfaces (*22*, *23*).

Needless to say, the cluster calculations have some limitations. In particular, the energy levels in the cluster are sharp, and the Cu-Mn molecular orbitals are nearly orthogonal to Cu $d_{x^2-y^2}$, because mixing is only due to weak spin-orbit coupling effects. The charge-transfer transition shown in Fig. 4 is therefore abrupt. In an extended system, the energy levels are broadened into bands, and the two bands near the Fermi level can be partially mixed. The charge carriers on interfacial Cu ions are thus generally expected to have mixed $d_{x^2-y^2}$ and $d_{3z^2-r^2}$ character, as experimentally observed. More elaborate ab initio calculations are required to obtain a quantitative description of the band dispersions at the interface. However, given the complexity of the unit cells and the broken translational symmetry at the interface, this presents a formidable challenge to current computational capabilities.

Concluding remarks. Our experiments show that the electronic structure of the CuO_2 layer is modified by covalent bonds across the interface. These results suggest that the orbital rearrangement and strong hybridization are at least partially responsible for the unusual magnetic behavior previously observed at cuprate-manganate interfaces (*22*, *23*) and contribute to the suppression of superconductivity near the interface (*13*). Further, the valence electrons of a large variety of transition metal oxides, whose properties in heterojunctions have been extensively investigated [including manganates (*24*), titanates (*25*), vanadates (*26*), ruthenates (*27*), and ferrites (*28*)], reside in nearly degenerate *d* orbitals and are hence subject to hybridization at interfaces.

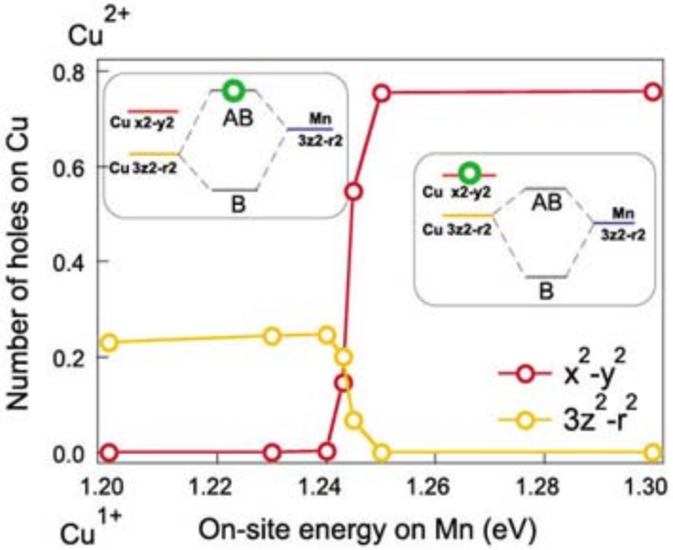

Fig. 4. Occupancy of Cu *d* orbitals at the LCMO-YBCO interface as a function of Mn hole on-site energy, as predicted by the exact-diagonalization calculations described in the text. The occupancy is given by the total number of holes, measured from the full-shell ($3d^{10}$) electron configuration. The corresponding formal Cu valence states are indicated for clarity. The insets show the orbital level scheme at the interface, including extended bonding (B) and antibonding (AB) "molecular orbitals" formed by hybridized Cu and Mn $d_{3z^2-r^2}$ orbitals. The hole is indicated as the green circle.

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Materials and Methods
Figs. S1 to S3
References

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Generation and Photonic Guidance of Multi-Octave Optical-Frequency Combs

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Ultrabroad coherent comb-like optical spectra spanning several octaves are a chief ingredient in the emerging field of attoscience. We demonstrate generation and guidance of a three-octave spectral comb, spanning wavelengths from 325 to 2300 nanometers, in a hydrogen-filled hollow-core photonic crystal fiber. The waveguidance results not from a photonic band gap but from the inhibited coupling between the core and cladding modes. The spectrum consists of up to 45 high-order Stokes and anti-Stokes lines and is generated by driving the confined gas with a single, moderately powerful (10-kilowatt) infrared laser, producing 12-nanosecond-duration pulses. This represents a reduction by six orders of magnitude in the required laser powers over previous equivalent techniques and opens up a robust and much simplified route to synthesizing attosecond pulses.

The generation of an ultrabroad and coherent spectrum spanning from the visible to extreme ultraviolet (XUV) and soft x-ray by high harmonic generation (HHG) in inert gases had led to the generation of attosecond pulses (*1*), enabling the measurement and control of previously inaccessible ultrabrief physical and chemical processes (*2*, *3*). Efforts are under way to circumvent the limitations of HHG on the conversion efficiency and the spectral location. Alternative techniques based on generation of higher-order stimulated Raman scattering (HSRS) in hydrogen or deuterium and the use of various driving conditions have been shown to exhibit a broad and coherent HSRS spectrum with a much larger photon-conversion efficiency and fewer constraints on the spectrum location (*4*–*7*). However, similar to the HHG method, they all come at the expense of either multiple driving pump lasers or ultrashort pulsed lasers and require a gigawatt level of peak powers in all cases.

Progress of relevance to nonlinear optics has also been made in developing photonic solutions based on gas confined in a hollow-core photonic crystal fiber (HC-PCF) (*8*). The ability of these fibers to confine together gases and light at mode areas on a scale of μm^2 while keeping them in interaction over length scales of several meters (*8*, *9*) has led to unprecedented low-light power–level nonlinearities in gas-phase materials (*10*) being accessed in compact systems (*11*). However, the generated spectra have been limited in bandwidth by the modest ~70-THz band gap width achievable in these fibers (*12*), which is much smaller than the ~1000-THz bandwidth required to generate attosecond pulses in the infrared-ultraviolet (IR-UV) domain.

We report on the generation of almost 1000-THz-wide HSRS comb-like spectra in molecular hydrogen confined to the core of a HC-PCF. This HC-PCF relies on a new photonic guidance whereby photons are confined not by photonic band gap but rather via a mechanism akin to Von Neumann–Wigner bound states within a continuum (*13*, *14*), in that the fiber-guided modes cohabitate with those of the cladding without notably interacting. This "inhibited interaction" between the hollow core–guided modes and the cladding continuum is explained by the high degree of the transverse-field mismatch between the core and cladding modes. This fiber is ideal for ultrafast optics because its transmission spans from the UV to mid-IR with low dispersion and relatively low loss. We have found that these properties enabled the generation of ultrabroad comb-like spectra through the use of transient stimulated Raman scattering (SRS) pumped by only a single laser with 12-ns pulse width, 1064-nm wavelength, and peak powers not exceeding 40 kW.

The HC-PCF investigated here is based on a Kagomé lattice (*8*). Although this HC-PCF was reported a few years ago, the underlying guidance mechanism was not understood. The fiber does not exhibit photonic band gap; instead, it was recognized that at the transmission wavelengths, the Kagomé lattice exhibits a lower density of photonic states (DOPS) (*9*). It would seem from these properties, which are summarized in fig. S1 (*15*) for a one-cell core Kagomé lattice HC-PCF (*16*), that guided core modes and cladding modes in a Kagomé lattice HC-PCF can coexist even at the same frequency $k\Lambda$ and effective index n_{eff}. This situation is illustrated by the near-field of the transmitted white light through a few centimeters of the fiber shown in fig. S1B. This indicates the presence of bound or quasi-bound states (here core modes) within a continuum (cladding modes). Even though such a situation is rare in physics and might seemingly contradict the very notion of a "bound" state, it is not unique. Electronic bound states at energies above the potential barriers were predicted by Von Neumann and Wigner as far back as the infancy of quantum mechanics (*13*, *14*). To shed light on the origin of this photonic quasi-bound state within a continuum, we investigated the nature of this continuum (i.e., the photonic states of the cladding structure) and their "inhibited interaction" with the HE_{11}-like core mode.

Figure 1A shows calculated core and cladding modes at representative frequencies of the two high-transmission bands of the fiber [i.e., band I and III; see also fig. S1D (*15*)]. The simulations show that, in the high-transmission bands, all cladding photonic modes with n_{eff} values close to that of the core mode are localized mainly at silica struts. They exhibit rapid field decay into the air regions, a very steep dispersion (n_{eff} variation with $k\Lambda$) (fig. S1G) and, more importantly, fast phase oscillations. The fast oscillations in glass are a manifestation of the large transverse wavevector components associated with such waveguide modes at low indices ($n_{eff} \leq 1$) and high $k\Lambda$. These cladding modes can be viewed as individual waveguide modes associated with the interconnected silica struts of the fiber microstructure. Indeed, the field distribution within the cladding region shows the same behavior, whether the cladding modes are calculated for an infinite structure without a core being present (see unit-cell mode in Fig. 1, A and B) or for a finite structure in which a core is included as shown in the second column in Fig. 1, A and B. Crucially, these features hold even for cladding modes that belong to the same symmetry class as a core mode and that have effective index values that differ by only $\Delta n_{eff} \sim 10^{-5}$ or less from the core-mode index. Despite this high degree of longitudinal phase matching, the hybridization is observed to be extremely weak over the broad frequency range of both bands I and III. This is illustrated in Fig. 1C by the low value of the power-in-glass fraction over these frequency ranges. Indeed, this value is below that of a photonic band gap fiber (*17*).

The weak interaction between the core and cladding modes is explained by the strong transverse-field mismatch between the modes, as evidenced by the fast field oscillations of the cladding mode, leading to the "washing-out" of the overlap with the slowly varying core-field distribution. This very weak overlap was also reported in (*18*), but no account of its origin was given. A further corroboration of the above scenario is the strong confinement of the field intensity within the core shown in Fig. 1D. The plot shows that the field has decayed by ~30 dB

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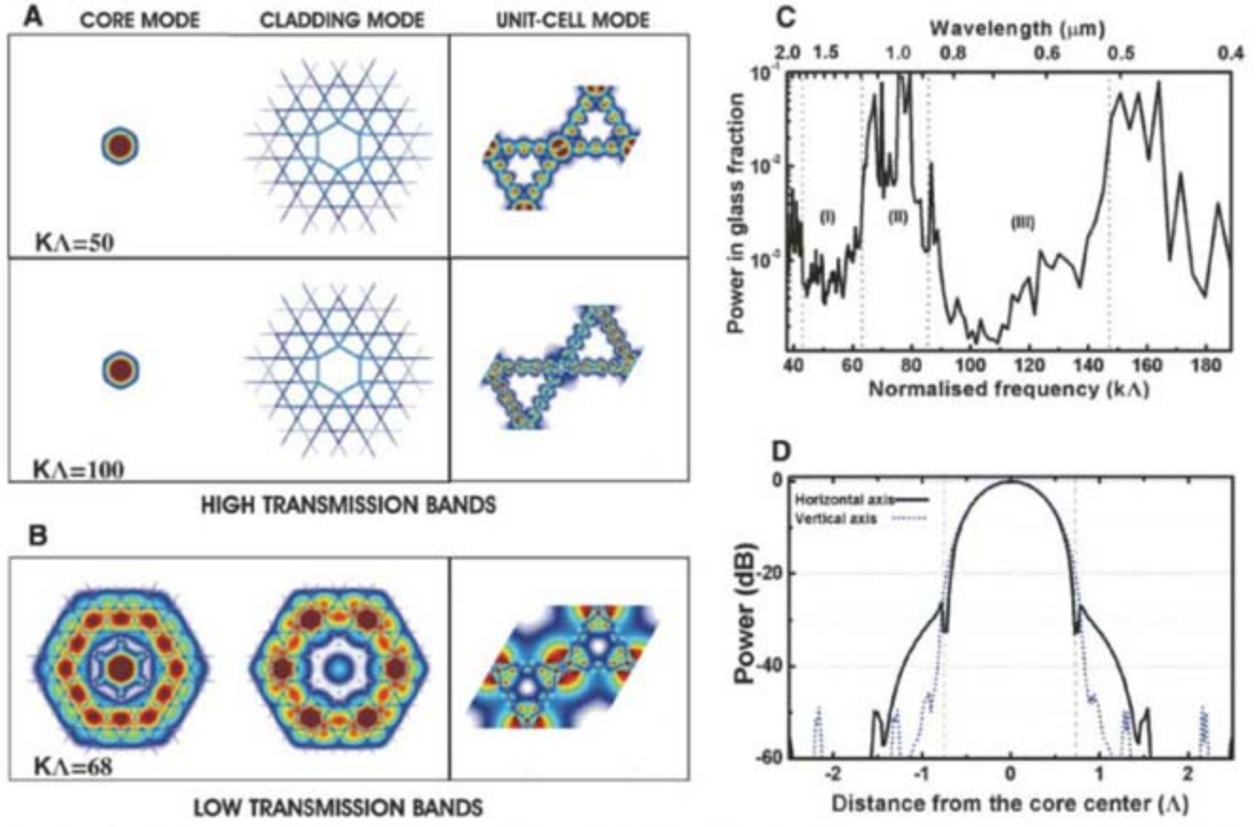


Fig. 1. Nature of the Kagomé lattice HC-PCF cladding modes. (**A**) The first two columns show the core mode and a cladding mode, respectively, for a frequency $k\Lambda = 50$ in band I (top) and $k\Lambda = 100$ in band III (bottom). At each frequency, the effective indices of the core and cladding mode differ by less than 10^{-5}. The third column shows the mode of an infinite Kagomé lattice, which corresponds to the cladding mode in the second column, calculated for the same frequencies at the Γ-point of the Brillouin zone. (**B**) Same as in (A) for $k\Lambda = 68$ in band II. (**C**) Power in the glass fraction plotted as a function of $k\Lambda$. (**D**) Intensity profile of HE_{11}-like mode along the horizontal and vertical cross-section, respectively; the orientation is the same as in (A) and (B). The vertical gray-dashed lines show the silica core-wall positions for the horizontal axis.

before the silica surround is reached. This figure is below the typical values, ~20 dB, calculated for band gap fibers with approximately the same mode size. Because so little field penetrates into the cladding, the core mode shows low propagation loss, which is also in agreement with experiment. Superficially, this "noninteraction" between states with the same symmetry might appear to violate the "noncrossing rule," but as was pointed out by Von Neumann and Wigner (*13*, *14*), this rule ceases to hold where dense continua are involved.

The fast-oscillating nature of the cladding modes also elucidates the immunity of the Kagomé HC-PCF to bend loss. In analogy with fibers that have a cylindrical symmetry, these modes are associated with a very high effective number, m, that governs the azimuthal phase variation of the field. A perturbation that may induce coupling between the core mode and such cladding modes necessarily requires a fast azimuthal variation (i.e., a large Δm). A fiber bend, however, is primarily associated with a change in m of just 1, thus providing the fibers with good resilience to bend loss. The coupling induced by such perturbations is also weakened owing to the small overlap between the core mode and cladding mode intensity distributions.

Figure 1B shows a representative mode ($k\Lambda = 68$) of the low-transmission band (band II). The modes of this band belong to a family of cladding modes for which the "inhibited interaction" with the core no longer holds (*15*). Those forming band II result from an interaction between the cladding hole modes (see the relatively flat red curve below the air-line in fig. S1G that extends up to $k\Lambda$~65 without interacting with the silica cladding modes) and glass strut modes, which occur near the lowest-order resonance wavelength given by $\lambda = 2t\sqrt{n_{gl}^2 - 1}$, where t is the strut thickness and n_{gl} the refractive index of the glass. This form of cladding mode is able to interact with the core mode, enhancing leakage loss from the core.

These theoretical findings open a new route to designing novel broadband HC-PCF where, instead of seeking photonic band gaps, one explores photonic structures that support photonic modes that exhibit a high degree of optical field orthogonality between the core and cladding components. Armed with this picture of guidance, we designed and fabricated a new Kagomé-lattice fiber in which the thickness of the glass struts is reduced to ~290 nm, but the pitch is maintained near 12 μm (Fig. 2, A and B). This has the effect of widening the high-transmission bands, thus maximizing the bandwidth for frequency generation and further reducing the dispersion. It also results in a shifting of the low-transmission band to ~590 nm (Fig. 2C) (*15*).

The new fiber shows low loss (typical loss is ~0.5 dB/m) at the wavelength of the pump laser

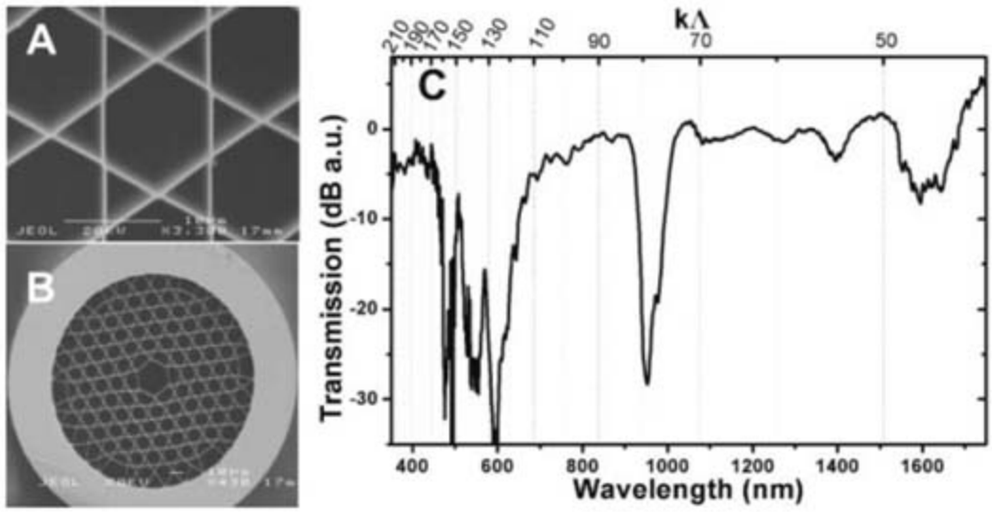


Fig. 2. (**A**) Scanning electron micrograph (SEM) showing the cladding structure of the bandwidth-optimized Kagomé HC-PCF and (**B**) lower-magnification SEM showing the full structure of the fiber used in the HSRS experiment. (**C**) Optical spectrum showing the optimized, broadband guidance of the fiber.

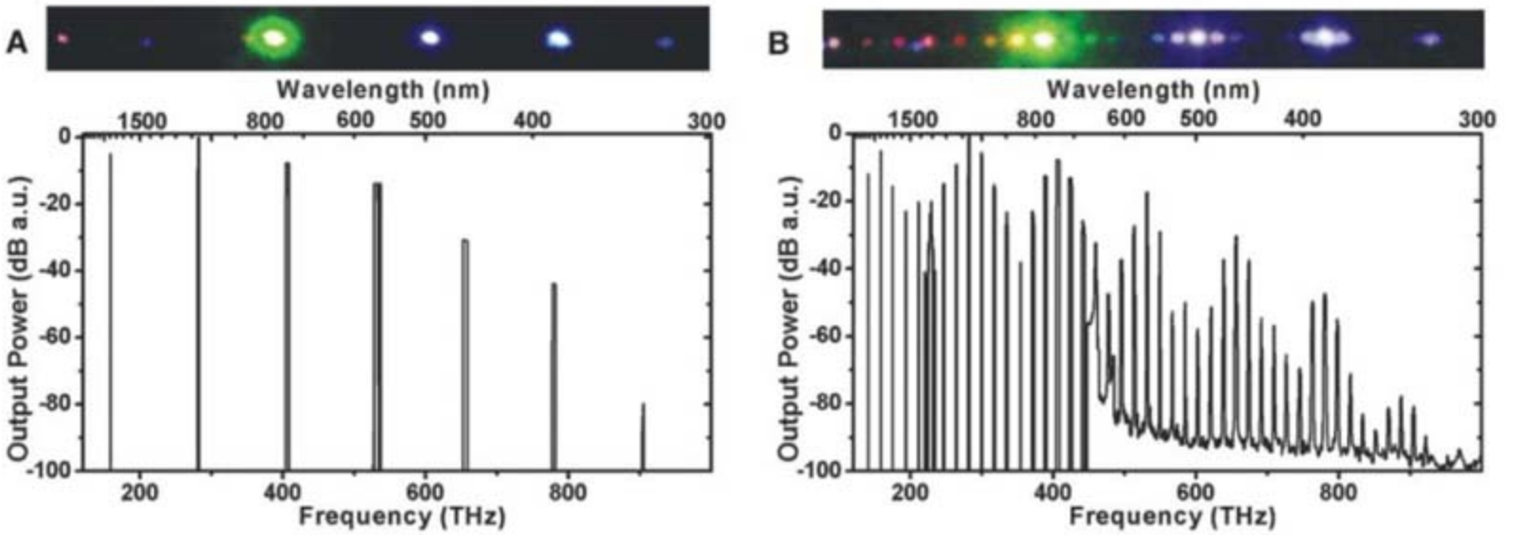
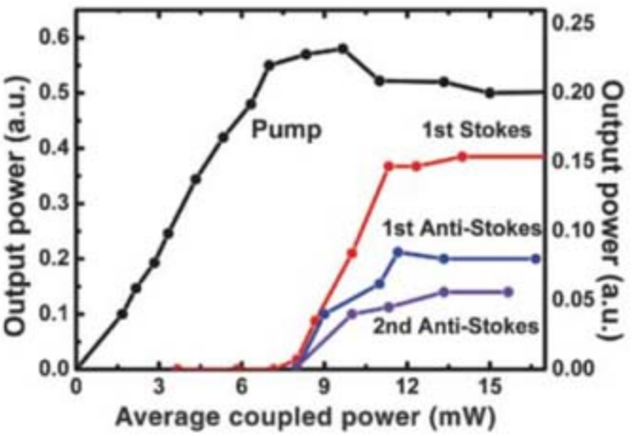


Fig. 3. Images and spectrum of the generated and transmitted HSRS through ~1-m-long hydrogen-filled Kagomé fiber for (**A**) a linearly polarized and (**B**) a circularly polarized laser input.

(i.e., 1064 nm) that is used to generate higher-order SRS spectra. SRS is a two-photon inelastic scattering of an incident laser beam by the molecular excitation of the Raman medium, resulting in frequency down-converted (Stokes line) or up-converted (anti-Stokes line) photons being shifted from the pump frequency by the Raman transition frequency Ω. Under appropriate driving conditions of the medium (*15*), a large number of mutually coherent high-order Stokes and anti-Stokes fields can be obtained over a very large frequency span (*4*–*7*, *19*). For example, it has been argued qualitatively that one could obtain a coherent HSRS spectrum with one pump laser, provided one operates in the transient and high-gain regime (*6*, *20*). Conventionally, this regime is limited to GW power, transform-limited subpicosecond lasers so that their pulse duration τ_p remains shorter than the dephasing time of the Raman medium T_2 (*6*, *20*). However, it was recently demonstrated that the long interaction length and the strong optical confinement offered by the HC-PCF extend the transient SRS regime to pulses much longer than T_2 and with low peak-power level (*21*). This is largely fulfilled in our experiment in which we used a 1-m length of the fiber described above filled with natural hydrogen at a pressure of 20 bars and excited by a Nd–yttrium-aluminum-garnet laser operating at 1064 nm and with τ_p = 12 ns (*15*).

Figure 3A shows a typical transmitted spectrum and spatial-mode images of some spectral lines, obtained when a 40-kW peak power of a linearly polarized pump laser is coupled into the fiber. In addition to the pump line, the spectrum contains six strong spectral lines consisting of one Stokes (~1892 nm) and five anti-Stokes components, the fifth anti-Stokes being in the UV (~332 nm). The lines are equally spaced by ~125 THz, which corresponds to the frequency of the vibrational Raman-transition $Q_{01}(1)$ of ortho-hydrogen. For the same pump power as above, when the laser polarization is changed from linear to circular in order to favorably excite the $S_{00}(1)$ rotational transition, a spectrum of 45 spectral components (Fig. 3B) can be observed. The spectrum consists of the vibrational HSRS components observed in Fig. 3A, with additional strong rotational sidebands on either side of them, spaced by the 18 THz of the $S_{00}(1)$ transition. The net result is a spectrum spanning from ~325 nm in the UV to ~2300 nm in the IR, with more than 53% of the input pump energy being converted to higher-order Stokes and anti-Stokes spectral lines.

Fig. 4. Detected output power from the pump (black circles and line), first Stokes (red circles and line), first anti-Stokes (blue circles and line), and second anti-Stokes components (purple circles and line), as a function of the coupled peak power. Each component is taken from a fraction of the output beam diffracted on a grating.



The observed spectra differ from the previously reported results obtained with HSRS in both the impulsive (*5*) and the transient regimes (*6*, *7*, *20*) by having far stronger Raman lines and a wider spectral span. This is achieved despite pumping with a longer wavelength and peak powers six orders of magnitude lower than with the above-mentioned techniques (*20*). Furthermore, compared to the spectra obtained in the adiabatic regime (*4*), the present ones exhibit comparable conversion efficiency and spectral bandwidth but with a peak pump power almost four orders of magnitude lower. Notably, this was obtained under conditions that satisfy neither the requirements for adiabatic preparation, because the SRS is seeded from the quantum noise, nor impulsive preparation, which requires laser pulses shorter than the period of the Raman molecular excitation.

For the sidebands to be suitable for sub-femtosecond pulse synthesis, they should be mutually coherent, as would be expected in the case of parametric wave-mixing processes. By contrast, in our case, the lines are initiated by spontaneous Raman scattering, raising the question of whether or not the lines are coherent. In this experiment, several indications point toward the mutual coherence that is desired. First, the parametric nature of the higher-order Raman sidebands is indicated by the observed simultaneous rise of all the spectral lines for both the vibrational and ro-vibrational spectra in accordance with (*20*). This is illustrated in Fig. 4, which shows the first Stokes (S1) and the first and second anti-Stokes (AS1 and AS2) of the vibrational transition all rising around a pump peak power of 13 kW. Another indicator of the parametric nature of the interactions is the observation of higher-order transverse spatial modes for some of the spectral lines, despite the fiber supporting a low-loss HE_{11}-like mode at the same wavelength (*15*).

Owing to the major role of the quantum noise in the initiation of the SRS process, we devel-

oped a new quantum analysis (i.e., SRS initiation from spontaneous emission) generalized to an arbitrary number of Stokes and anti-Stokes (S/AS) lines to investigate the mutual coherence of these sidebands (*15*). The results, applied to an isolated vibrational (or rotational) Raman transition, predict that in the high-gain transient regime, all S/AS sidebands carry phases that are automatically correlated in a deterministic manner (i.e., phase-locked). Indeed, even though the spontaneous seed for the S1 pulse is temporally and spatially randomly fluctuating during the generation process, this noise is heavily filtered out under the high-gain transient conditions to leave out a Stokes field with a single overall phase. Concomitantly with the generation of S1, there is a rise of a first anti-Stokes (AS1) field and a molecular excitation, which are determined by the same phase that arises spontaneously (*22*, *23*). This result is ascertained by calculating the degree of mutual coherence between the Stokes and anti-Stokes fields (*15*).

More importantly, the theory shows that both the higher-order S/AS pairs and the molecular excitation retain the same key features of sharing a common phase related to that of the first S/AS pair. The observed high efficiency of the Raman conversion indicates that we were able to excite substantial molecular coherence even though it was initiated from quantum noise. In analogy with the adiabatic regime (*4*), the results of the new theory can be viewed as the generation of a coherent molecular spatial excitation, which modulates the first S/AS pair by adding S/AS sidebands. These then become modulated to generate sidebands of their own, and so on.

The spectrum generated with circular polarization contains both rotation- and vibration-generated lines. Consequently, there is a second coherent molecular excitation, implying the introduction of one additional random phase. This in itself does not destroy the phase coherence of either the pure vibrational or the pure rotational spectra, but to experimentally produce sub-fs pulses, these degrees of freedom need to be relatively controlled to correct for this additional random phase. Also, one could optimize the medium pressure to suppress completely the vibrational lines (*6*), leaving only the mutually coherent rotational lines.

The extension of using this HC-PCF in other Raman excitation regimes is straightforward. For example, its combination with the adiabatic preparation technique would enhance the conversion efficiency and further reduce the required pumping powers involved while ensuring better control over the spectral components' phases. This would enable the generation and synthesis of attosecond pulses with much lower pumping powers. Furthermore, in addition to the intrinsic fundamental importance of the discovery of this "Von Neumann–Wigner"–related waveguidance to photonics, it will provide us with new tools to develop next-generation HC-PCFs with even broader bandwidth and lower transmission loss. For example, with lower loss figures, one could synthesize ultrashort pulses using continuous-wave pumps. This would permit the synthesis of arbitrary optical waveforms with a degree of control approaching that in electronics.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5853/1118/DC1
Methods
SOM Text
Figs. S1 to S7
References

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Engineering Entropy-Driven Reactions and Networks Catalyzed by DNA

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Artificial biochemical circuits are likely to play as large a role in biological engineering as electrical circuits have played in the engineering of electromechanical devices. Toward that end, nucleic acids provide a designable substrate for the regulation of biochemical reactions. However, it has been difficult to incorporate signal amplification components. We introduce a design strategy that allows a specified input oligonucleotide to catalyze the release of a specified output oligonucleotide, which in turn can serve as a catalyst for other reactions. This reaction, which is driven forward by the configurational entropy of the released molecule, provides an amplifying circuit element that is simple, fast, modular, composable, and robust. We have constructed and characterized several circuits that amplify nucleic acid signals, including a feedforward cascade with quadratic kinetics and a positive feedback circuit with exponential growth kinetics.

The development of modular biochemical circuit elements poses several challenges. First, distinct signals must be carried by distinct chemical species, motivating the use of information-carrying molecules whose sequences can be used to encode signal identity. Second, "wiring up" a gate to specified inputs and outputs involves the design and synthesis of new molecules; this calls for modular gate designs. Third, a fast and robust catalytic mechanism must be identified and coupled to a suitable energy source in order to create gates with signal gain. Fourth, it must be possible to construct circuits of arbitrary complexity that can produce an unlimited variety of dynamical behaviors. Finally, there should be no leak or crosstalk between distinct signals and gates. It is difficult to meet all these challenges simultaneously.

Nucleic acids are attractive for this purpose because the combinatorial sequence space allows for an enormous diversity of signal carriers, and the predictability and specificity of Watson-Crick base pairing facilitate the design of gate architectures. The "RNA world" hypothesis further suggests that sophisticated biochemical organization can be achieved with nucleic acids alone (*1*), and nucleic acids have indeed been shown to be a versatile construction material for engineering molecular structures and devices (*2*, *3*), including catalytic (*4*–*8*) and logical (*9*–*12*) control elements and circuits (*13*–*17*). Engineering (deoxy)ribozyme-based logic gates has been very effective, resulting in systems containing over 100 gates operating independently in parallel (*10*) as well as systems demonstrating cascading of a signal between two gates (*13*, *15*, *16*). Alternatively, hybridization-based systems, usually driven by the energy of base-pair formation, have proven especially suitable for cascading signals, as demonstrated by a circuit five layers deep (*17*). That work, relying primarily on noncatalytic logic gates, identified amplification and signal gain as essential for scaling up to large cascaded circuits. We provide a solution to this problem.

The entropy-driven catalytic gate presented here is substantially simpler than previous hybridization-based designs; moreover, it is faster, better understood, and more modular. The net reaction is shown in Fig. 1A: Fuel strand (*F*) reacts with the three-stranded substrate complex (*S*), displacing output and signal strands (*OB* and *SB*) from linker strand (*LB*) to form waste complex (*W*). The total number of base pairs in the reactants and products is unchanged; the reaction is driven forward thermodynamically by the entropic gain of the liberated molecules. Fuel, signal, catalyst, and output are all single-stranded DNA molecules that can be of similar lengths; thus, each molecule may play multiple roles within a network. For example, the output of one gate may serve as the input to another. Notably, catalyst *C* and output *OB* may be entirely independent in sequence (*18*); this modularity implies that a catalytic gate can be designed to act at any point within a preexisting circuit. Unlike previous hybridization-based catalyst systems, the reaction design does not require unusual secondary structures such as pseudoknots and kissing loops. Undesired interactions can be avoided by design (*19*–*21*), resulting in reliable and predictable circuit behavior.

Strands are conceptually subdivided into functional domains (number labels in Fig. 1) whose sequences determine the pattern of interactions between circuit components. [Domain sequences are given in Table 1; see supporting online material (SOM) text S1 for design details.] The domains can be grouped by purpose: domains 3 and 5 are termed toehold domains, whereas domains 1, 2, 4, and 6 are termed specificity domains. Toehold domains are short enough to bind only fleetingly in the absence of additional binding (and need not be distinct), but they greatly accelerate the initiation of strand displacement reactions (*22*). Specificity domains ensure specific interactions [even a single mismatch can slow down branch migration substantially (*23*)] and determine the identities of the catalyst and output molecules. The lengths of the toehold domains determine kinetics and need to be between roughly 4 and 10 nucleotides (nt), but the specificity domains may be of any length sufficient to ensure thermal stability. Domains 1 and 6 of *OB* and *SB*, respectively, are inert, whereas their respective toeholds are sequestered in *S*.

Catalytic activity has two characteristic behaviors: the speedup of the target reaction and the re-release of the catalyst to allow for multiple turnover. To achieve these behaviors, we introduce and apply a design principle that we call toehold exchange (Fig. 1B): *C* first binds to the single-stranded toehold domain $\overline{5}$ on *S* to form the four-stranded intermediate *I1*, which then rearranges (by branch migration) to form *I2*. The binding between toehold domains 3 and $\overline{3}$ is too weak to keep *SB* attached, so *I2* spontaneously dissociates into *SB* and *I3*. Newly exposed $\overline{3}$ then facilitates the binding of *F*, resulting in *I4*, which then quickly rearranges to release *OB* and *I5*. Finally, *I5* rearranges so that *C* is attached only by the binding of 5 and $\overline{5}$, which spontaneously dissociates to leave *W* and regenerate *C*. The reaction mechanism presented here, based on branch migration and driven by entropy, differs from the traditional view of catalysis in biological organisms in that it requires no enzymes and alters no covalent bonds.

It is important to ensure that alternative interactions do not interfere with intended gate functions. Toward this end, a key design principle is that the complements of the specificity domains never appear in their single-stranded

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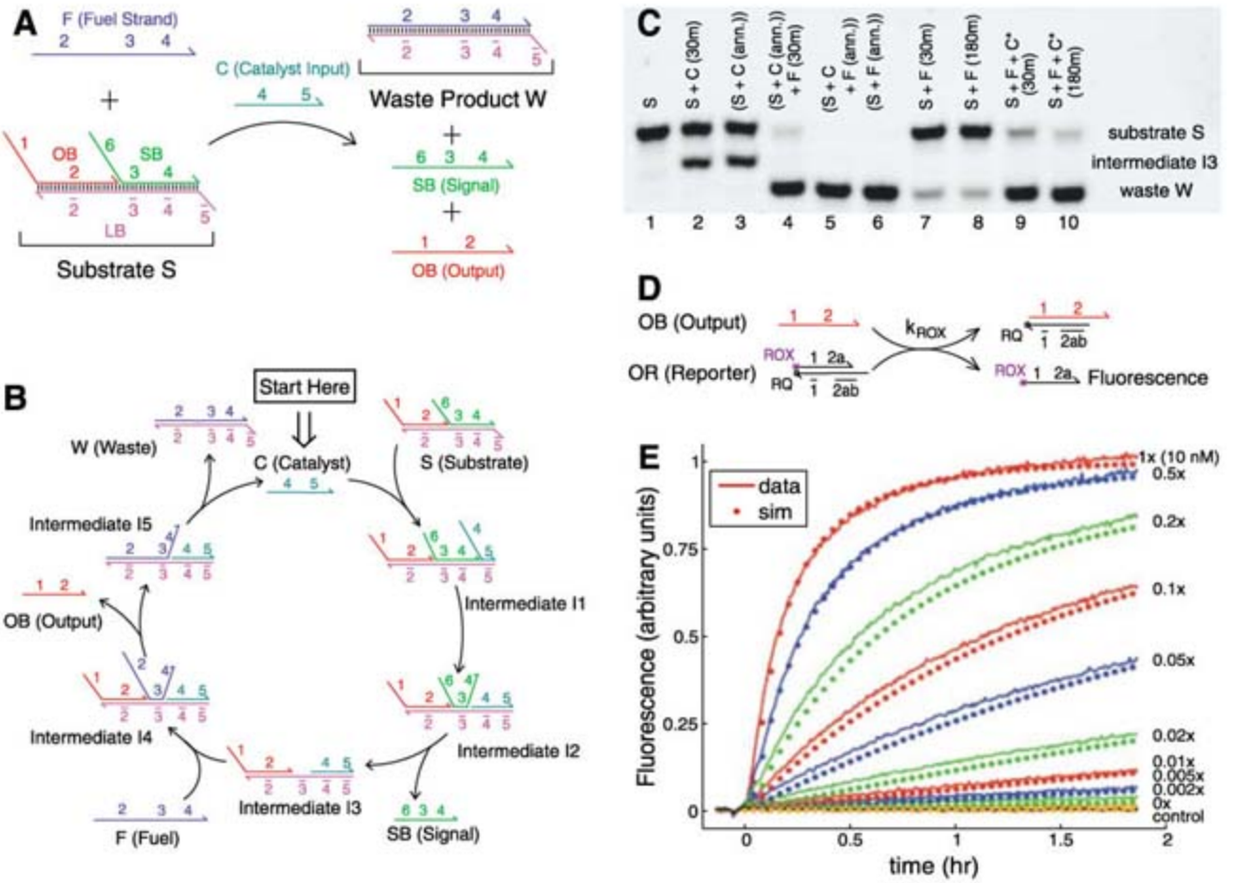


Fig. 1. The entropy-driven reaction. (**A**) System components. Number labels denote functional domains, which are continuous stretches of DNA that act as units in binding. Domain $\overline{x}$ is the complement of (and will hybridize to) domain *x*. (**B**) The proposed catalytic pathway. Reverse reactions are also present and modeled (with the exception of *I5*+*OB*→ *I4*, which occurs at a negligible rate). (**C**) Analysis by PAGE (12% native gel) of the reaction mechanism. Unless otherwise noted, all experiments were performed at 25°C in tris-acetate (TE) buffer supplemented with 12.5 mM $MgCl_2$. Here, [*S*] = [*F*] = 200 nM. [*C*] = 200 nM, except where *C** denotes 20 nM. "ann." denotes that species were annealed; "30 m" denotes that the reaction occurred for 30 min. See fig. S5 for the full gel, including control lanes. (**D**) Fluorescent reporter strategy. ROX denotes the carboxy-X-rhodamine fluorophore, and RQ denotes the Iowa Black Red Quencher. Domain 2 is subdivided into 2a, 2b, and 2c; 2ab consists of 2a and 2b (Table 1). (**E**) Demonstration of catalysis. Different amounts of *C* were introduced into the system at $t \approx 0$. Here, [*S*] = 10 nM = 1×, [*F*] = 13 nM, and [*OR*] = 30 nM. Fluorescence (in all figures) is reported in units such that 0.0 is the background fluorescence of the quenched reporter and 1.0 is the fluorescence of ~10 nM of triggered reporter. The control trace (black) shows the reaction with no substrate *S* and no catalyst *C*. Dotted lines show curves calculated with the reduced reaction model. sim, simulated.

form. Except at toeholds, no two molecules interact with each other via complementary single-stranded domains. The catalytic gate is therefore expected to function for most choices of domain sequences lacking strong secondary structure and spurious mutual interactions (*19*–*21*).

In Fig. 1C, polyacrylamide gel electrophoresis (PAGE) is used to verify the catalytic pathway (*24*). By reacting substrate *S* (purified by gel) and catalyst *C* in the absence of fuel *F*, we prevent the reaction from progressing past intermediate *I3*. The amount of *I3* produced after 30 min (lane 2) is almost identical to that present at equilibrium, as assessed by annealing the reaction components (lane 3). This suggests that all reactions up to *I3* are fast on this time scale. Similarly, the subsequent reaction between *I3* and *F* is also fast (lanes 3 to 5). The complete system behaves as expected: The uncatalyzed reaction is slow (lanes 7 and 8), and a substochiometric quantity (0.1×) of *C* enables the reaction to proceed rapidly to near-completion (lanes 9 and 10).

In order to measure the time course of the catalyzed reaction by means of a fluorescent reporter without interference from fluorophore-quencher interactions (*25*) (SOM text S3), we use an indirect reporter complex *OR*. *OR* reacts stochiometrically with output *OB* to separate a fluorophore-labeled strand from a quencher-labeled strand, thereby increasing fluorescence (Fig. 1D). The rate constant for the reporter system was measured to be $k_{ROX} = 4 \cdot 10^5 M^{-1}s^{-1}$ (SOM text S4). Because initial [*OR*] = 30 nM is in excess to [*S*] = 10 nM, the reporter complex remains substantially in excess, and the reporting delay should remain less than 100 s, which is short as compared to the half time of the catalyzed reactions. *OR* does not react substantially with *S*, because there are no single-stranded toeholds to initiate interaction. Measurements of the kinetics of the catalyzed reaction over a 500-fold range of catalyst concentration are shown in Fig. 1E.

We modeled this system using the reduced reaction set shown below.

$$S + F \xrightarrow{k_0} SB + OB + W$$

$$S + C \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} I3 + SB$$

$$I3 + F \xrightarrow{k_2} I5 + OB$$

$$I5 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C + W$$

$$OB + OR \xrightarrow{k_{ROX}} \text{Fluorescence}$$

where $k_0 = 2.3 \cdot 10^1 M^{-1}s^{-1}$,

$k_1 = 6.5 \cdot 10^5 M^{-1}s^{-1}$,

$k_2 = 4.2 \cdot 10^5 M^{-1}s^{-1}$,

$k_3 = 4 \cdot 10^{-3} s^{-1}$ (fitted), and

$k_{ROX} = 4 \cdot 10^5 M^{-1}s^{-1}$

The first reaction shown models the uncatalyzed (leak) reaction. Intermediate steps in branch-migration reactions are omitted, because they are relatively fast at experimental concentrations (SOM text S5) (*26*) and because intermediates *I1*, *I2*, and *I4* are not observed in PAGE analysis of reactants and products (Fig. 1C). Noting the approximate symmetry between the corresponding reactions, we assume that $k_{-3} = k_1$ and $k_{-1} = k_2$. The rate constants k_0, k_1, and k_2 were measured individually (fig. S4); k_3 was fit to the data of Fig. 1E. The time course of the catalyzed reaction over a wide range of catalyst concentrations is accurately reproduced by this reduced system of rate equations (Fig. 1E). According to this model, the addition of catalyst can accelerate the reaction by over four orders of magnitude ($k_2/k_0 = 1.8 \cdot 10^4$).

In the net reaction, each base pair that is broken is replaced by another of the same type, so the net free energy change from base-pairing interactions should be small. The reaction is driven by the gain in configurational entropy corresponding to the liberation of *OB* and *SB* at the cost of localizing *F*. To confirm the dominance of this entropic driving force, we truncate *F* by removing up to 8 nt from its 5′ end, making the products more and more thermodynamically disfavored. Nonetheless, in all cases

Table 1. Domain sequences of basic catalytic reaction.

Domain	Sequence	Length (nt)
1	5′-CTTTCCTACA-3′	10
2a	5′-CCTACG-3′	6
2b	5′-TCTCCA-3′	6
2c	5′-ACTAACTTACGG-3′	12
3	5′-CCCT-3′	4
4	5′-CATTCAATACCCTACG-3′	16
5	5′-TCTCCA-3′	6
6	5′-CCACATACATCATATT-3′	16

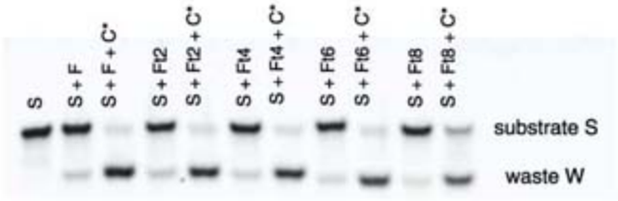


Fig. 2. Verification of entropic driving force. Analysis by PAGE (12% native gel) of reactions with truncated fuel strands. [*S*] = [*F*] = 200 nM. [*C*] = 20 nM, as denoted by the asterisk. All reactions were run at 25°C for 3 hours. "Ft2" denotes that two bases were truncated from the 5′ end of fuel strand *F*.

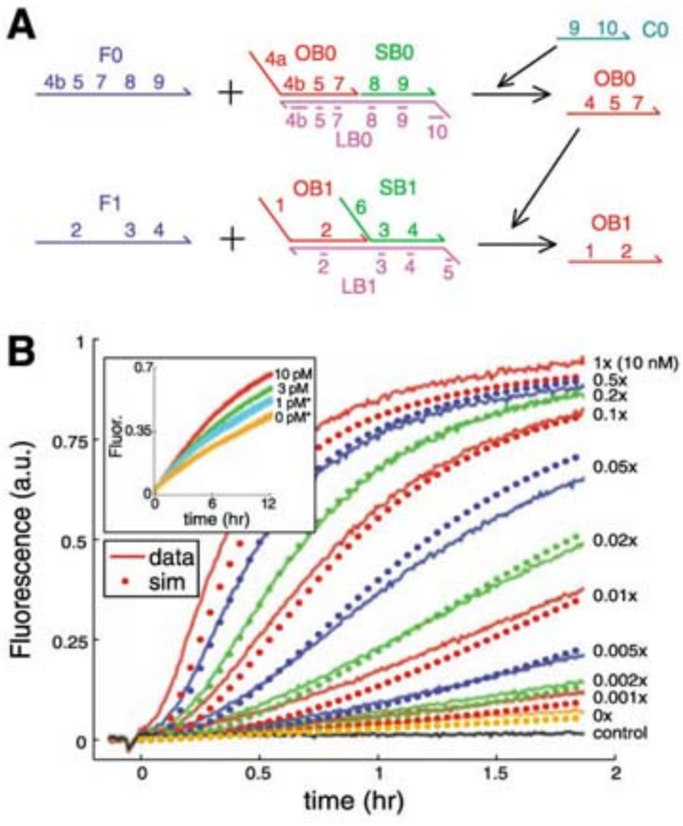


Fig. 3. A two-layer cascaded network. (**A**) Schematic. See table S2 for sequences of new domains. (**B**) Kinetics. Indicated amounts of initial catalyst *C*0 were added at $t \approx 0$. Fluorescence derives from reporter complex *OR* (Fig. 1D) at 30 nM. Dotted lines show simulated traces; see SOM text S8 for details on reaction rates and modeling. a.u., arbitrary units. (Inset) Response to 0.0010×, 0.0003×, and 0.0001× catalyst. The asterisk indicates that three independent reaction traces are shown. 1.0 fluorescence units correspond to ≈ 10 nM of triggered reporter.

the waste product is favored at equilibrium (Fig. 2; see SOM text S6 for discussion on entropy and free energies). The thermodynamic driving force, being dominated by center-of-mass configurational entropy of released molecules, is somewhat robust to environmental conditions such as temperature and salt concentrations that alter the strength of DNA hybridization (SOM text S7 and fig. S6).

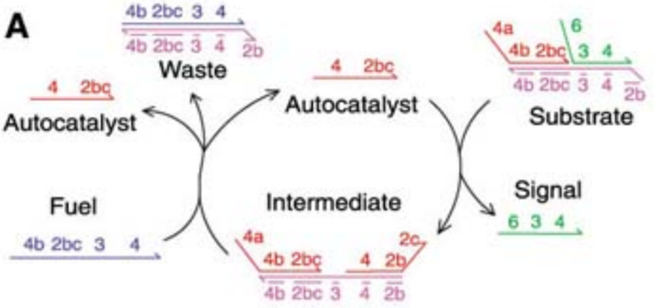
To demonstrate cascaded circuit construction, we designed a two-layer feedforward network by introducing an upstream catalyst system whose output acts as the catalyst for the original system (Fig. 3A). For clarity, *F*, *OB*, and the other reactants and products from Fig. 1 are relabeled *F*1, *OB*1, and so forth. Catalyst *C*0 catalyzes the production of *OB*0 (which contains a subsequence identical to *C* from Fig. 1), which in turn catalyzes the production of *OB*1. The concentration of upstream catalyst *C*0 is constant, so initially [*OB*0] increases linearly with time, which causes [*OB*1] to increase quadratically with time (Fig. 3B). Eventually, the substrates and fuels are depleted, and the reaction halts, giving rise to an overall sigmoidal shape to the fluorescence traces (Fig. 3B). The model previously used can be extended to predict the behavior of this feedforward circuit data (SOM text S8).

This cascaded system can be used as an amplifier to detect small quantities of *C*0. Repeated fluorescence experiments show that we are able to distinguish reliably between 1 pM (0.0001×) catalyst *C*0 and 0× catalyst within 12 hours (Fig. 3B, inset). This corresponds to a roughly 900-fold amplification of the input signal. (1 pM of catalyst triggered ≈ 900 pM of reporter above the baseline set by the 0× reaction.) For comparison, 1 pM corresponds to about one molecule per eukaryotic cell volume. Repeated measurements of independent samples show less than 3% variability across all timepoints (SOM text S9).

Feedback is another important feature of both biological regulatory networks and artificial control circuits. Exponential growth kinetics can be achieved by redesigning the reaction presented in Fig. 1 such that output *OB* contains catalyst *C* as a subsequence (Fig. 4A). The reaction is then autocatalytic. Figure 4C shows the time course of this reaction for a wide range of catalyst concentrations. In a process dominated by initial exponential growth ($c \approx c_0 e^{\lambda t}$), the time to reach a threshold degree of completion depends logarithmically on the initial concentration c_0 (where c is the concentration of the exponentially growing species, λ is the characteristic time constant, and t is time). Thus, a linear trend in a log-linear plot of initial concentration to time to half completion ($t_{1/2}$) is indicative of exponential growth. [Such plots are used as calibration standards for quantitation methods such as real-time polymerase chain reaction (PCR) (*27*).] Fig. 4D shows that our autocatalytic system has this characteristic behavior, implying that exponential growth kinetics have indeed been achieved and that the reaction is not substantially affected by product inhibition. Further confirmation comes from the quality of fit to the data of a model based on rate constants derived for the catalyst system of Fig. 1 (SOM text S8).

We also demonstrate feedback in a two-layer circuit by redesigning *OB*1 so that it can, in turn, catalyze the *F*0 + *S*0 reaction (SOM text S11). Feedback in this cross-catalytic system causes the concentrations of both *OB*0 and *OB*1 to grow exponentially at early times.

Largely because of their relevance to the origin of life and to the RNA world (*1*), autocatalytic and cross-catalytic self-replication reactions have been proposed and demonstrated previously (*28*). However, such systems typically suffer from product inhibition and thus exhibit parabolic, rather than exponential, growth kinetics. Recent exceptions include cross-catalytic deoxyribozymogens (*13*) and catalyzed self-assembly (*29*) based on the hybridization chain reaction (*14*); our autocatalyst system is faster than either of these. Reducing the spontaneous activity of the circuit (for example, by improved purification of the substrate complex) is an important goal for increasing the sensitivity to



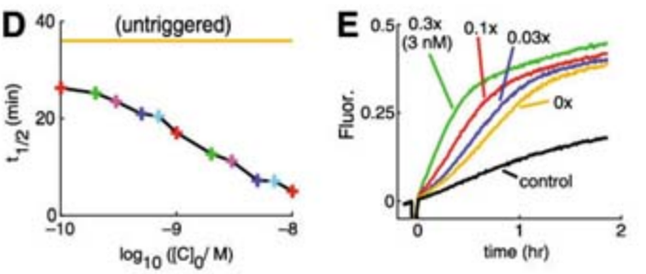


Fig. 4. The autocatalyst system. (**A**) Proposed reaction pathway. See table S2 for sequences of new domains. (**B**) Reporter complex *SR*, used for monitoring autocatalytic and cross-catalytic reaction networks. TET denotes the tetrachlorofluorescein fluorophore. FQ denotes the Iowa Black Fluorescent Quencher. (**C**) Kinetics of autocatalysis. Indicated amounts of autocatalyst were added at $t \approx 0$. At 30 min, 1% (100 pM) was amplified 25-fold over the untriggered reaction. Reporter *SR* was present at 20 nM = 2×. Control sample (black) contained no fuel *F*. (**D**) Semi-log plot of $t_{1/2}$ (in minutes) as a function of logarithm of molar trigger concentration. The orange line shows $t_{1/2}$ of the untriggered reaction. (**E**) Performance in the simultaneous presence of total RNA and unfractionated cell lysate. Total RNA present in solution was 10× (by mass) that of the sum of all relevant catalyst DNA. Active cell lysate, as would be used in an in vitro translation system, was added to be 1.1% by volume of total reaction (17 μL in 1500 μL). The control reaction did not contain any substrate *S*. Experiments involving total RNA but not cell lysates did not show the observed drift.

the point that our autocatalyst could be used as an enzyme-free constant-temperature alternative to PCR for detecting known sequences.

For many applications in biotechnology, nucleic acid devices must remain functional in the presence of naturally occurring macromolecules. We therefore tested the autocatalyst system in the presence of an excess of mouse liver total RNA with rabbit reticulocyte lysate (Fig. 4E). Reactions proceeded to apparent completion with no more than a twofold slowdown, and the presence of a 3% trigger can still be detected.

The ability to construct larger circuits will enable the wide range of chemical circuit functions needed for sophisticated applications. Our entropy-driven catalytic reaction networks are suited for scaling up to larger circuits. The modular molecular design makes synthesis of more complex components and networks with arbitrary topology straightforward. To demonstrate this, we constructed an entropy-driven catalytic analog AND gate in which both of two catalysts are required to release output (SOM text S12 and fig. S11). For scaling up to large circuits, independent catalyst systems must have negligible crosstalk. The success of quantitative models that assume no crosstalk, as presented above, is encouraging; further evidence comes from a test of two independent catalyst systems operating in the same solution (fig. S12). Finally, catalytic systems have the potential to avoid the slowdown that plagued previous attempts to construct large nucleic acid circuits (*17*).

Future nucleic acid control circuits must be interfaced to molecular sensors and actuators. This may be achieved directly when the inputs and outputs are themselves nucleic acids, such as for the detection, analysis, and response to complex nucleic acid samples (*9*, *30*) or for the control of nucleic acid nanomachines (*2*, *31*). Nucleic acid circuits can also respond to and control more general chemical events: In principle, the release of an oligonucleotide could regulate covalent chemistry by controlling (deoxy)ribozyme activity (*9*) or reactant proximity (*32*). Additionally, signals carried by small organics and other non–nucleic acid molecules can be read by nucleic acid systems with the use of aptamer domains (*33*, *34*) and other binding interactions that can regulate toehold accessibility (*35*, *36*). Thus, nucleic acids could provide a general-purpose system for the synthesis of embedded control circuitry within aqueous chemical systems.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5853/1121/DC1
SOM Text S1 to S12
Figs. S1 to S12
Tables S1 to S4
References

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Radar Sounding of the Medusae Fossae Formation Mars: Equatorial Ice or Dry, Low-Density Deposits?

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The equatorial Medusae Fossae Formation (MFF) is enigmatic and perhaps among the youngest geologic deposits on Mars. They are thought to be composed of volcanic ash, eolian sediments, or an ice-rich material analogous to polar layered deposits. The Mars Advanced Radar for Subsurface and Ionospheric Sounding (MARSIS) instrument aboard the Mars Express Spacecraft has detected nadir echoes offset in time-delay from the surface return in orbits over MFF material. These echoes are interpreted to be from the subsurface interface between the MFF material and the underlying terrain. The delay time between the MFF surface and subsurface echoes is consistent with massive deposits emplaced on generally planar lowlands materials with a real dielectric constant of ~2.9 ± 0.4. The real dielectric constant and the estimated dielectric losses are consistent with a substantial component of water ice. However, an anomalously low-density, ice-poor material cannot be ruled out. If ice-rich, the MFF must have a higher percentage of dust and sand than polar layered deposits. The volume of water in an ice-rich MFF deposit would be comparable to that of the south polar layered deposits.

Units of the Medusae Fossae Formation (MFF) occur discontinuously at equatorial latitudes along the boundary of the hemispheric dichotomy from Amazonis to Elysium Planitiae (~130°E to 240°E) (*1*, *2*). The MFF may be among the youngest surficial deposits on Mars, unconformably overlying ancient Noachian heavily cratered highlands and young Amazonian lowlands (*1*–*8*). However, pedestal craters on the outer edge of the MFF

deposits have been cited as evidence of an older age (*9*). The local topographic relief of the MFF units varies greatly, reaching a maximum of more than 3.5 km (*5*–*7*). The morphology of the MFF units is complex and variable. Over large horizontal scales (tens of kilometers), the undulating hills of the MFF are relatively smooth (Fig. 1). At smaller scales, many of the MFF units are marked by systems of parallel ridges and grooves interpreted as yardangs (*10*–*14*) (Fig. 2). Remnant yardangs and outliers some distance from the thicker units suggest that MFF deposits once covered a larger area of the northern lowlands (*3*, *6*) (Fig. 2A). Layering is observed in the MFF deposits that varies in scale from coarse, indurated layers that cap weaker, more friable material to thin, pervasive layering (*3*, *4*, *6*, *15*–*17*).

A variety of origins have been proposed for the MFF deposits. These include ignimbrite or volcanic ash deposits from now-buried vents (*1*, *2*, *6*, *11*), eolian deposits from materials weathered early in martian history (*1*, *18*), and deposits analogous to polar layered and circumpolar deposits formed either as a consequence of polar wandering (*9*) or during periods of high obliquity (*7*) [see supporting online material (SOM) text]. Units of the MFF are associated with the "Stealth" region on Mars (SOM text), so named because no echo is detected in 3.5- and 12.6-cm Earth-based radar data (*19*–*21*).

We report here on observations of the MFF deposits by the MARSIS radar sounder (*22*) (SOM text). Subsurface echoes are detected that correspond to the basal interface between the MFF material and the underlying plains material. We also characterize the thickness and electrical properties of the MFF deposits as a guide to their bulk porosity and/or ice fraction.

MARSIS data obtained between March 2006 and April 2007 cover all the units of the MFF (Fig. 1). Radargrams, or time-delay renderings of the sounding data along the spacecraft ground track, show subsurface echoes, offset in time-delay from the surface return, where the tracks cross the MFF (Fig. 3). The subsurface echoes generally parallel the surface return except near the margins where, in some cases, the subsurface and surface echoes converge (Fig. 3). The observed time-delay in the radargrams is consistent with the expected depth to the interface between the MFF deposits and the underlying terrain.

The westernmost MFF deposits form low-relief, undulating hills (Fig. 1) and overlie relatively young (Late Amazonian-aged) lowlands volcanic plains associated with Cerberus Fossae (*2*, *8*) (Fig. 2A). The inferred elevation of the subsurface interface corresponds closely with the floor of a valley separating two hills where MFF material has been almost completely stripped away, nearly exposing the Cerberus plains (Fig. 2B and Fig. 3A). The MFF material that forms Lucus Planum is deposited on older (Hesperian-aged), lowlands knobby terrain (*2*, *8*) (Fig. 1). The interface beneath the eastern flank of this unit is flat and largely continuous (Fig. 3B). The MFF material exposed in the pronounced valley of Medusae Fossae itself extends from the northern lowlands into the ancient heavily cratered (Noachian-aged) southern highlands, locally burying the dichotomy boundary and the cratered highlands (*2*, *8*) (Fig. 1). A generally flat, continuous subsurface interface that extends for several hundred kilometers is separated in time-delay from a shallower, discontinuous interface associated with a layer internal to the MFF deposits (Fig. 3C). The subsurface echo from the eastern flank of Eumenides Dorsum is more spread out in time-delay but appears to delineate the north-downward slope of the buried dichotomy boundary (Fig. 3D). MFF material overlying Amazonian-period volcanic plains (*1*, *8*) forms the prominent ridges of Amazonis Mensa and Gordii Dorsum (Fig. 1). There are two parallel subsurface echoes from the valley between the ridges (Fig. 3E) that correspond to the base of the MFF material and an internal dielectric horizon. The discontinuous subsurface echoes associated with the northern tip of Gordii Dorsum correlate in time-delay with the basal echo from the valley floor (Fig. 3E, far right in radargram). The easternmost MFF deposits overlie the dichotomy boundary and Amazonian volcanic plains of Olympus Mons and the Tharsis Montes (*1*) and narrow northwestward into a ridge (Fig. 1). A discontinuous subsurface reflection from beneath the western part of the ridge suggests a flat basal interface (Fig. 3F).

Previous analyses suggested that some MFF units are draped over preexisting topographic rises in the lowlands (*13*, *23*). The subsurface interfaces revealed by MARSIS suggest that MFF materials are deposited on generally planar materials in the northern lowlands and the downward slope of the dichotomy boundary (Fig. 3).

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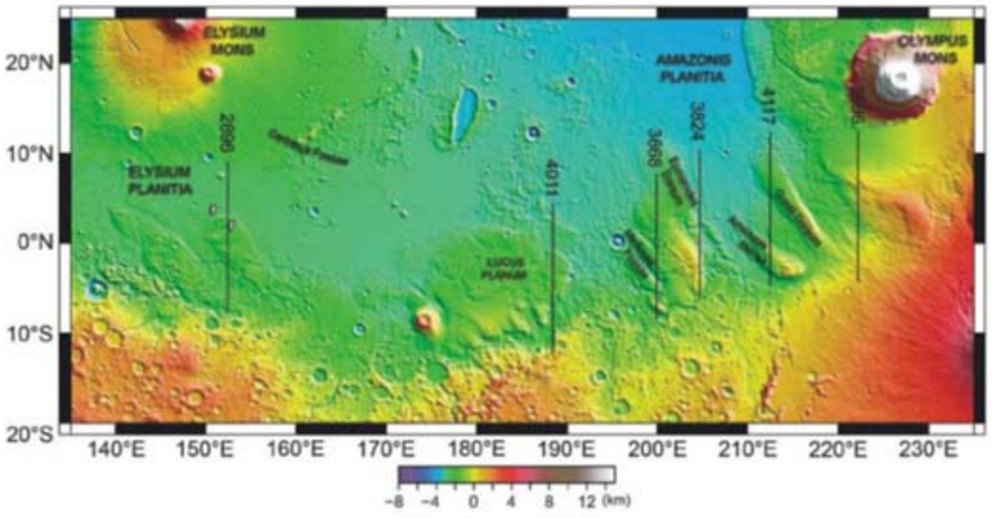

Fig. 1. The Medusae Fossae Formation in Elysium and Amazonis Planitiae along the dichotomy boundary. The locations of MARSIS orbit tracks 2896, 4011, 3868, 3824, 4117, and 3996 are indicated by back lines (from left to right, respectively) overlaid on Mars Orbiter Laser Altimeter (MOLA) color-coded shaded relief. The locations of Thermal Emission Imaging System (THEMIS) images shown in Fig. 2 are indicated by the small white rectangles.

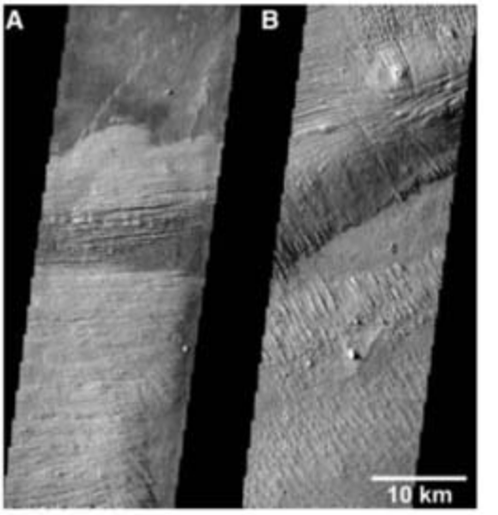

Fig. 2. High-resolution THEMIS images of the MFF material in Elysium Planitia. (**A**) THEMIS visible image (frame #V05275021) shows an outlier of MFF material that is being stripped, partially exposing the underlying lowlands plains. (**B**) THEMIS visible image (frame #V13163010) shows numerous yardangs and a valley stripped of MFF material. The locations of images (A) and (B) are to the left and right, respectively, of orbit track 2896 shown in Fig. 1.

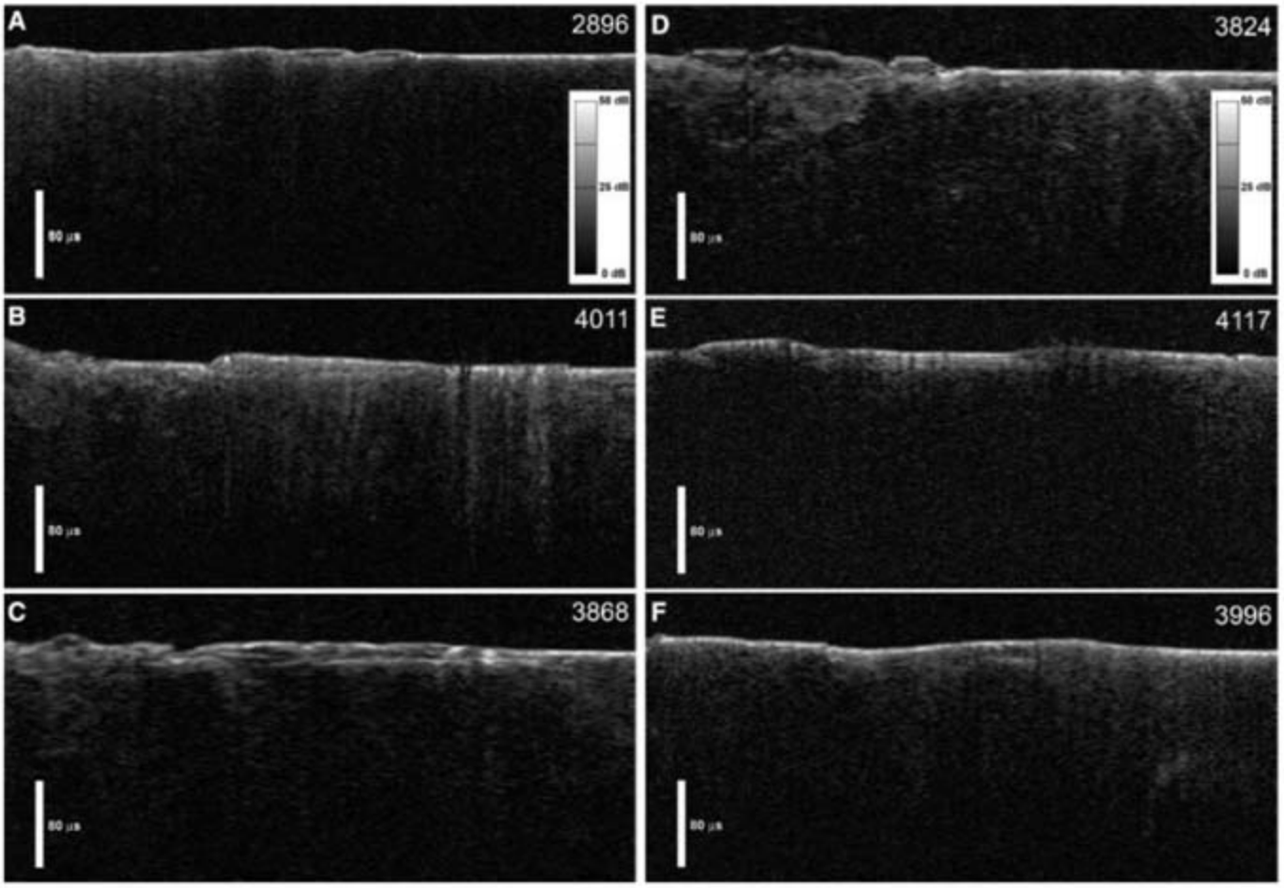

Fig. 3. Radargrams showing MARSIS data for orbit 2896 (**A**), 4011 (**B**), 3868 (**C**), 3824 (**D**), 4117 (**E**), and 3996 (**F**). Echoes are plotted in time-delay versus position along the orbit. The subsurface echoes are offset in time-delay from the surface echo and are interpreted to be nadir reflections from the interface between the MFF deposits and the lowland plains material. The peak surface return is corrected to agree with the MOLA topography along the orbit track. The radargrams are resampled to a uniform along-track length of ~1000 km. All the orbits are ascending except for orbit 2896.

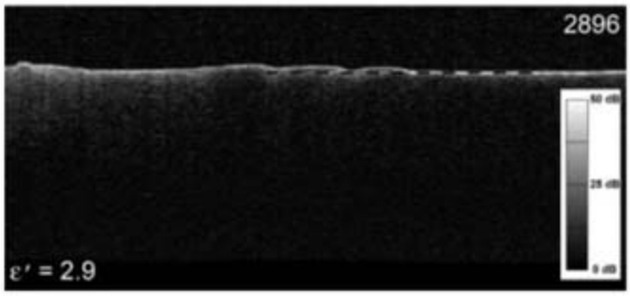

Fig. 4. Radargram showing MARSIS data for orbit 2896 converted to depth using a dielectric constant $\varepsilon' = 2.9$ for the MFF material. The dashed black line shows the projection of the lowland plains beneath the MFF deposits. There is good agreement between the basal reflector and the projection of the exposed surrounding plains (compare with Fig. 3A).

MARSIS data support estimates of the total volume of MFF material calculated using apparent base-level elevations in the lowlands. These estimates range from 1.4×10^6 km^3 (*4*) to 1.9×10^6 km^3 (*6*).

MARSIS observations provide an opportunity to evaluate the electrical properties of the MFF where the material is deposited on lowlands plains that are exposed nearby (Fig. 2A). The observed time delays in the MFF deposits correspond to a bulk real dielectric constant ε' of ~2.9 ± 0.4 (SOM text and fig. S1), based on the projection of the surrounding plains beneath the material (Fig. 4). A variation in ε' of 2.5 to 3.3 does not result in a large range in the radar-predicted thickness because h is a function of $\sqrt{\varepsilon'}$. The dielectric properties of a material are related to its density and composition. The real part of the dielectric constant is modulated strongly by density. The imaginary component of the dielectric constant ε'' and the loss tangent, $\tan\delta = \varepsilon''/\varepsilon'$, are strongly influenced by target composition. Radar losses due to attenuation in the deposits were estimated using the method outlined in Porcello *et al.* (*24*). At 4 MHz center frequency (Band 3), we obtain losses of ~0.0048 ± 0.0024 dB/m (SOM text, fig. S2). For ε' of 2.9 and a center frequency of 4 MHz, these losses correspond to a range in loss tangent of ~0.002 to 0.006 (SOM text).

MARSIS studies of the PLD (*22*, *25*) suggest a ε' value of about 3, consistent with pure water ice, based on the agreement between the inferred depth of the basal interface and the projection of the surrounding surface. The loss tangent of the PLD is estimated to range from <0.001 to 0.005 (*22*, *25*). Our analysis suggests a similar real dielectric constant (2.5 to 3.3) and a comparably low range of loss tangent (0.002 to 0.006) for the MFF materials. The loss tangents derived for the MFF deposits are below the range measured for terrestrial volcanic materials (*26*) but comparable to some low-titanium lunar materials (*27*). Thus, our first-order estimates of the dielectric losses span a range that includes some dry, unconsolidated geologic materials and mixtures of pure water ice and sediment. The real dielectric constant of the MFF and PLD deposits is also low relative to the behavior of compacted rock-derived materials, which are well fit by a function of the form $\varepsilon' = 1.96^d$, where d is the density in g/cm^3 (*26*). A maximum ε' of 3.3 corresponds to an average density of about ~1.8 g/cm^3, which is low for the expected self-compaction of 0.5 to 2.5 km of a dry geologic material.

There are two plausible interpretations of these observations. The first is that the MFF material is poorly consolidated and comprised of non-ice material with low dielectric loss. If the MFF material is an ice-poor ash or eolian deposit, it must have an unusually high porosity and low bulk density at depths up to 2.5 km to account for the estimated values of ε'. MFF deposits with a depth-averaged bulk density >1.9 g/cm^3 will have an ε' value outside the measured range.

The second possibility is that the MFF material is ice-rich, with a non-ice component of higher real dielectric constant and loss tangent (ice present as a minor component within a matrix of $\varepsilon' = 6$ does not match the observed properties). The extensive fields of yardangs in the MFF deposits, landforms that occur in variably indurated to poorly consolidated material that is easily eroded by wind (*1*–*3*, *5*, *6*, *15*), suggests that sublimation must have removed volatiles from the putative ice-rich deposits to

leave meters of dust and sand. The accumulation of meters of sediments suggests that the non-ice component of an ice-rich MFF deposit may be larger than the maximum 10% estimated for the south polar layered deposits (SPLD) (*25*). This, in turn, suggests a higher modeled real dielectric constant than that of pure ice.

Although the real dielectric constant and dielectric losses may be consistent with an ice-rich material, the existing data do not exclude the possibility that the MFF deposits are an anomalously low density, ice-poor material. In either case, these deposits appear to have unique characteristics from other martian deposits studied to date by radar sounding. An ice-rich MFF raises the intriguing possibility of a large volume of water ice in the equatorial zone of Mars beneath a veneer of dust and sand. MARSIS observations suggest that the total volume of ice in the SPLD is ~1.6×10^6 km^3 (*25*). If the MFF deposits are ice-rich, estimates of their total volume suggest a volume of water comparable to that in the SPLD.

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Three-Dimensional Splay Fault Geometry and Implications for Tsunami Generation

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Megasplay faults, very long thrust faults that rise from the subduction plate boundary megathrust and intersect the sea floor at the landward edge of the accretionary prism, are thought to play a role in tsunami genesis. We imaged a megasplay thrust system along the Nankai Trough in three dimensions, which allowed us to map the splay fault geometry and its lateral continuity. The megasplay is continuous from the main plate interface fault upwards to the sea floor, where it cuts older thrust slices of the frontal accretionary prism. The thrust geometry and evidence of large-scale slumping of surficial sediments show that the fault is active and that the activity has evolved toward the landward direction with time, contrary to the usual seaward progression of accretionary thrusts. The megasplay fault has progressively steepened, substantially increasing the potential for vertical uplift of the sea floor with slip. We conclude that slip on the megasplay fault most likely contributed to generating devastating historic tsunamis, such as the 1944 moment magnitude 8.1 Tonankai event, and it is this geometry that makes this margin and others like it particularly prone to tsunami genesis.

Most of Earth's largest and most destructive earthquakes and tsunamis occur along the global belt of subduction zones (*1*–*4*). Great (moment magnitude > 8.0) earthquakes are generated when large areas of the subduction megathrust rupture, a process that often generates large tsunamis such as those produced by the 2004 Sumatra and 2006 Java earthquakes (*5*, *6*). The size and destructive power of tsunamis that often accompany great subduction earthquakes is determined largely by the amount and area of vertical uplift of the sea bed, and these factors are especially sensitive to the geometry of the slipping fault as the earthquake rupture approaches the sea floor (*7*–*9*). Very long thrust faults that rise from the plate boundary megathrust and intersect the sea floor along the lower slope of the margin—known as out-of-sequence or megasplay faults and recently identified as first-order features in the Nankai Trough (*10*, *11*)—are also common in other subduction zones such as Alaska (*12*, *13*), Sunda (*14*), and Colombia (*15*). These megasplay faults have been hypothesized to efficiently transfer displacement to the near surface, fostering tsunami genesis, but owing to the lack of resolution of the shallow structure of these faults, the capability of the megasplay in enhancing tsunami generation has been controversial (*16*, *17*). Moreover, an earthquake that ruptures up to (or near) the surface (i.e., one with a slip distribution skewed to the updip end) has an enhanced tsunamigenic potential (*18*).

The Nankai Trough is characterized by destructive earthquakes that occur repeatedly along the plate boundary megathrust (*19*). A large out-of-sequence thrust (OOST), first recognized as a strong seismic reflection (*10*), branches from the megathrust fault ~50 km landward of the trench south of Kii Peninsula, where it forms the trenchward boundary of Kumano Basin (Fig. 1). Swath-bathymetric and seismic reflection data show a pronounced, continuous outer ridge of topography that extends more than 120 km along the strike (Figs. 1 and 2) and is related to the splay fault slip. This fault, termed the "megasplay" (*11*), is a fundamental structural element of the margin. Substantial long-term slip is documented by sequence boundaries and progressive landward tilting of the strata in Kumano Basin (*3*).

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Moreover, the megasplay separates rocks with considerably higher seismic velocity on its landward side from rocks of lower seismic velocity toward the trench (*20*), indicating that it represents a major mechanical discontinuity and leading Wang and Hu (*21*) to hypothesize that the megasplay is the boundary between two distinct structural wedges: (i) a stronger "inner wedge" on its landward side overlying the seismogenically active part of the megathrust and (ii) a weaker "outer wedge" on the seaward side overlying the aseismic frontal décollement.

Many authors have speculated that coseismic slip is concentrated along the Nankai megasplay (*8*, *10*, *16*, *17*), but earthquake and tsunami inversions lack the resolution to distinguish between slip that has been diverted up the megasplay and slip along the frontal décollement segment of the plate boundary megathrust. What has been missing is evidence that the megasplay has recently been active and may thus have been responsible for the tsunami caused by the 1944 Tonankai earthquake. Mechanical arguments further suggest that the megasplay has participated in great subduction earthquakes (*21*, *22*). Wang and Hu (*21*) proposed that the accretionary prism (the outer wedge) can be described as a "dynamic Coulomb wedge," whose compressive deformation intensifies at the time of a great earthquake because of coseismic velocity-strengthening of the aseismic frontal décollement. The strengthening of the frontal décollement promotes slip upward along the megasplay.

Here, we present the results of two- and three-dimensional (2D and 3D) seismic reflection surveys across the Nankai Trough subduction complex south of central Honshu, Japan (Fig. 1). These new seismic data, acquired using a commercial seismic vessel towing four hydrophone streamers and two airgun source arrays (table S1), image the subduction plate boundary megathrust and the subsidiary megasplay (Figs. 2 to 4 and movie S1) that have caused great earthquakes and tsunamis in the past (*17*, *23*). The streamers recorded the reflected seismic wave field generated from the active seismic source directed into the sub–sea floor, and this wave field was then migrated to form the seismic images with 3D prestack time migration (table S1), typical for industrial seismic imaging (*24*). These images reveal the scale, continuity, and degree of geologically recent near-surface activity of the megasplay fault system, supporting the

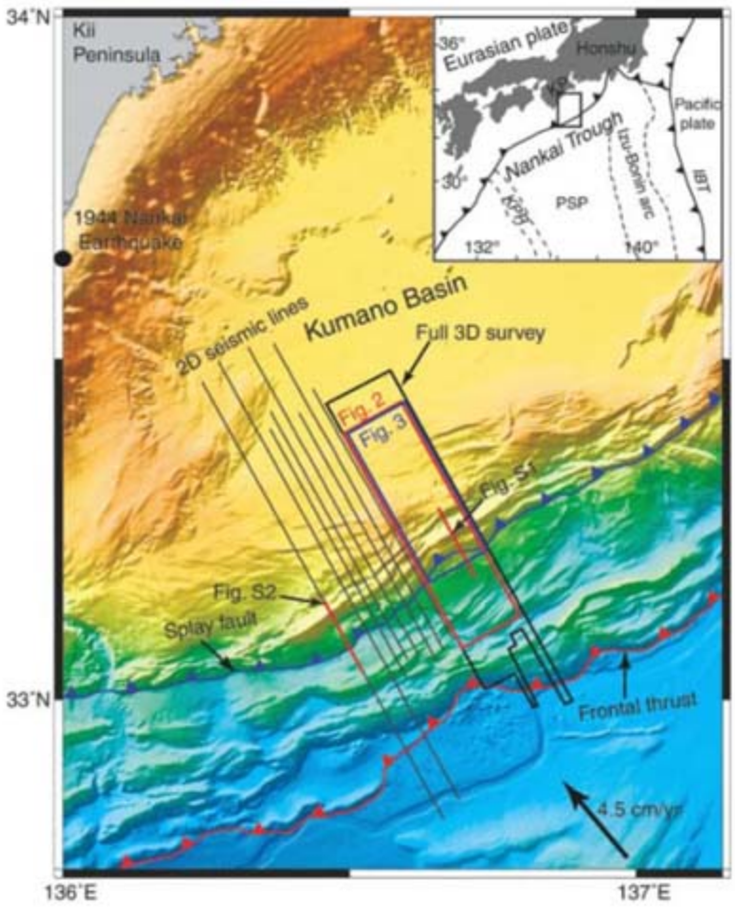

Fig. 1. Location map showing the regional setting of the Nankai Trough (upper right inset). PSP, Philippine Sea Plate; KPR, Kyushu-Palau Ridge; IBT, Izu-Bonin Trench; KP, Kii Peninsula. Convergence direction between the Philippine Sea Plate and Japan is shown at the lower right.

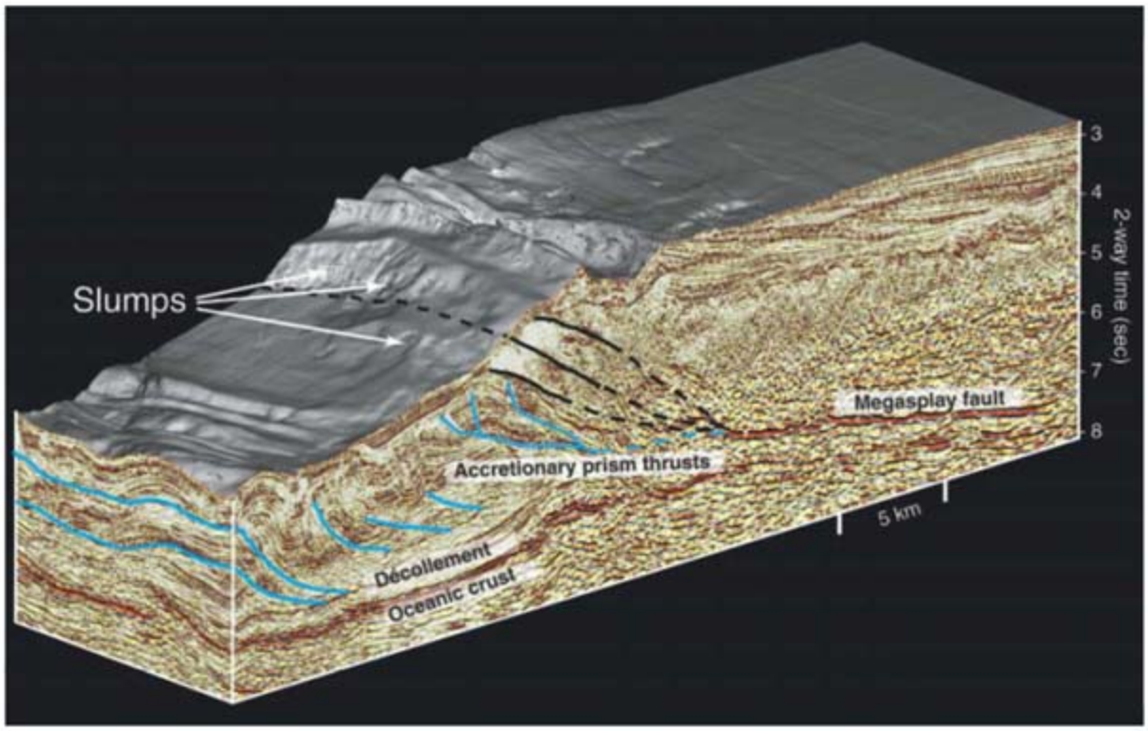

Fig. 2. 3D seismic data volume depicting the location of the megasplay fault (black lines) and its relationship to older in-sequence thrusts of the frontal accretionary prism (blue lines). Steep sea-floor topography and numerous slumps above the splay fault are shown.

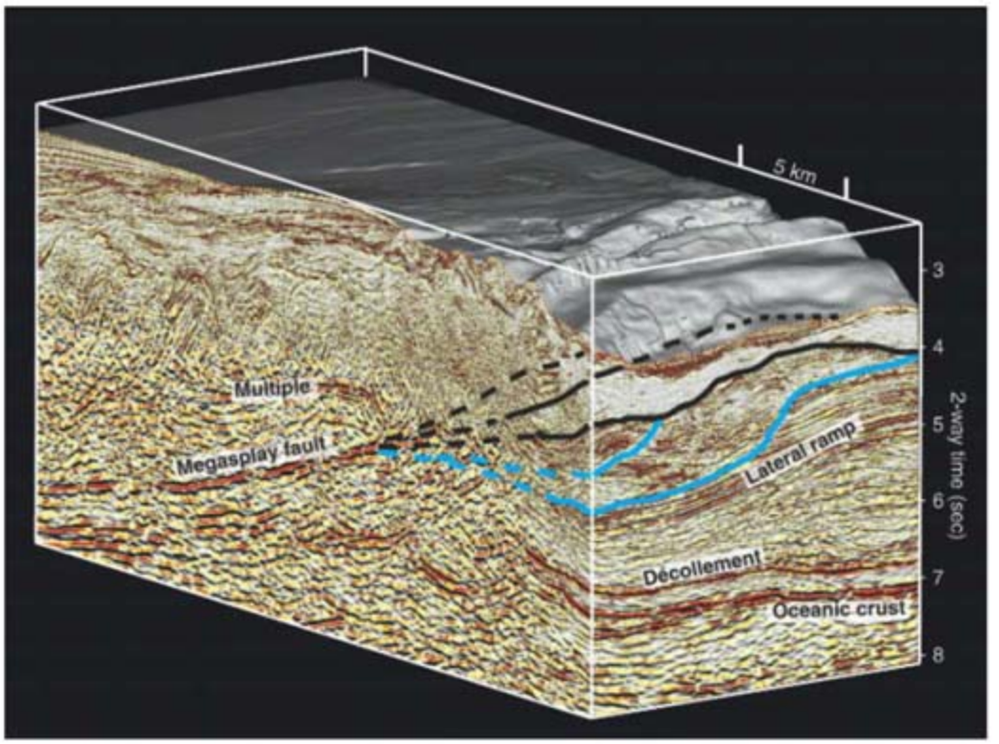

Fig. 3. 3D data volume showing relations of in-sequence thrusts of the frontal accretionary prism (blue lines) and the younger out-of-sequence branches of the splay fault (black lines). The top of a thrust sheet that has been folded above a lateral ramp in the frontal prism is cut off by the younger megasplay fault.

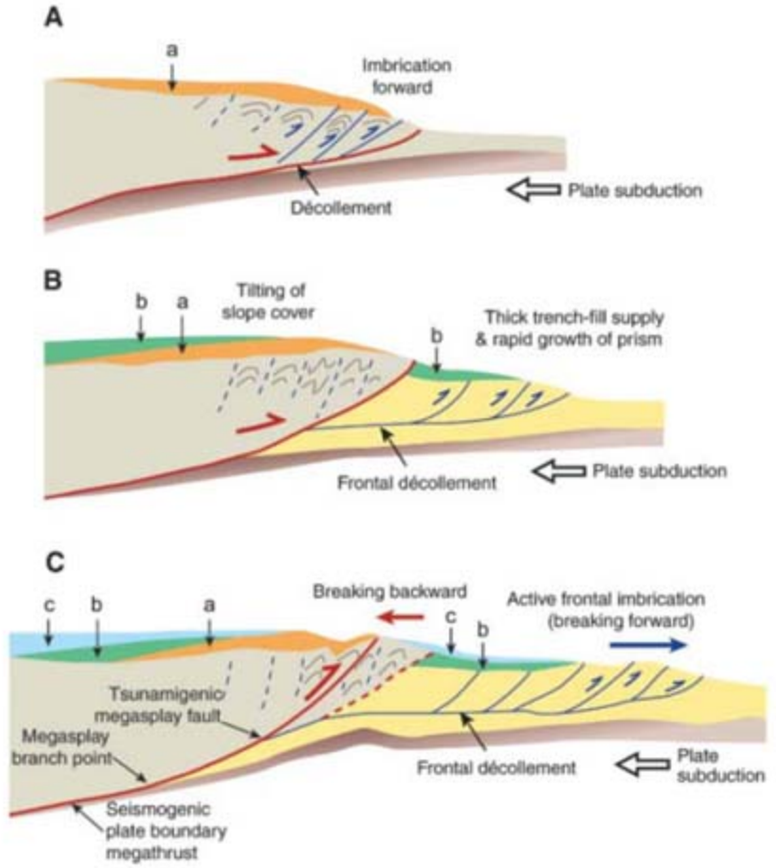

Fig. 4. (**A** to **C**) Summary diagram showing the development of the Nankai accretionary prism in the Kumano Basin area. After "normal" in-sequence thrusting and building of an accretionary prism, an out-of-sequence (splay) fault system broke through at the back of the prism. a, b, and c refer to sequential sedimentary sequences.

hypothesis that it is a first-order feature and a key part of the coseismic, tsunamigenic slip system. This detailed picture of the megasplay offers a key to link slip within the megathrust seismogenic zone up to the sea floor, the likely locus of tsunami generation. Our data thus demonstrate the connection between the regional megasplay fault that originates within the shallow portion of the seismogenic zone and the near-surface region. Slip directed along the megasplay to the sea floor, or near to it, could have generated the 1944 tsunami (Figs. 2 and 3).

The 3D geometry of the megasplay system shows that it has two branches, with the upper branch being younger (Fig. 2). Both branches truncate (and hence are younger than) the thrust faults within the accretionary prism. The top of the landward-most thrust package of the frontal accretionary zone (Figs. 2 and 3 and fig. S1) is cut off by the lower splay fault (Fig. 4B). This thrust package is deformed over a lateral ramp (Fig. 3), a branch off of an underlying thrust that dips to the southwest, perpendicular to the main direction of thrusting. If the overlying fault were older than the underlying fault, it would be carried "piggyback" on the underlying thrust and would also have been deformed by motion up the lateral ramp, but it is not. This observation further indicates that the thrusts are breaking backward (away from the deformation front) (Fig. 4C) in an OOST mode (*25*), rather than breaking forward (toward the deformation front) in an in-sequence mode that dominates the frontal part of this and other accretionary prisms (Fig. 4, A and B). In addition, a substantial amount of sediment has accumulated on top of the underlying thrust sheet and has been overridden by the advancing splay fault (fig. S1), indicating that the underlying thrust sheet was inactive before being overridden by the splay fault. Furthermore, the higher splay fault is clearly younger than the lower fault, because it has not yet overridden a substantial amount of slope sediment and the overall slope sediment cover is thinner than that covering the older fault.

Direct fault intersections with the sea floor are rare; however, a portion of a 2D line southwest of the 3D survey area illustrates clear propagation of the splay fault to the sea floor (fig. S2). This fault connects laterally to the lower splay fault shown in fig. S1, perhaps indicating that slip along the lower fault is still occurring in the southwest region. Thus, the fault does not everywhere propagate all the way to the surface; however, the displacement of this older thrust block during a great earthquake would generate a large tsunami, even without a sea-floor break (*9*).

A key to understanding the generation of tsunamis during great earthquakes is determining exactly where slip is apportioned on the splay/frontal décollement system. The amount of motion along the megasplay may control the magnitude of tsunami generation, and we presented several lines of evidence to indicate that the megasplay system is actively accommodating an

appreciable component of plate boundary motion. The most compelling evidence connecting the megasplay to the recent tsunamigenic slip is the geographical coincidence with the updip termination of slip during the 1944 Tonankai event, as inferred from tsunami (*17*, *26*) and seismic (*27*) waveform inversions and recent structural studies. These studies all suggest that the megasplay may have experienced coseismic slip, so our discovery of recent movement along a strand of the megasplay system is strong evidence that lends support to this interpretation.

Inversion of earthquake seismicity data shows that rupture in the Tonankai earthquake initiated near the downdip end of the slip area and propagated updip (*23*). Inversion of tsunami data (*17*) cannot adequately distinguish contributions to wave generation from interplate slip along the décollement or along the splay fault during the Tonankai earthquake, but results favor slip along the splay fault. Because of the modeling techniques employed, the vertical resolution of the updip extent of both coseismic slip and tsunami source area is relatively poor. The horizontal resolution is better, however, and the slip inversions suggest that rupture did not propagate to the trench but terminated close to where the megasplay intersects the surface (*17*, *23*). As rupture approached the surface, it could have (i) continued along the basal décollement, dying out in soft sediments of the outer accretionary wedge, or (ii) propagated up the megasplay. Kame *et al.* (*22*) modeled this scenario using an elastodynamic fault formulation and concluded that coseismic slip on the megasplay branch is favored over the basal décollement and that simultaneous slip on both is unlikely. This mechanical argument, combined with the tsunami source modeling and our current observations, suggests that the megasplay thrust system is presently a part of the "plate boundary fault," as defined by megathrust earthquake rupture (Fig. 4C).

We have shown that the most active fault in the prism is the megasplay and that there is less activity on the frontal décollement. This splay fault represents one single fault that is continuous from the deep seismogenic zone up to the surface. This observation supports the suggestion by Wang and Hu (*21*) that the velocity-strengthening behavior of the frontal décollement causes the slip to concentrate along the megasplay during great earthquakes. As the mechanical boundary between the inner and outer accretionary wedge (*21*), the megasplay could thus be seen as producing a (deformable) backstop for the outer wedge. Major slip along a fault with the geometry of the megasplay during great earthquakes thus increases the potential for tsunamis and explains why this and some other margins foster tsunami generation whereas still others do not.

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Supporting Online Material

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Figs. S1 and S2
Table S1
Movie S1

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Rise and Fall of Species Occupancy in Cenozoic Fossil Mollusks

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In the time between speciation and extinction, a species' ecological and biogeographic footprint—its occupancy—will vary in response to macroecological drivers and historical contingencies. Despite their importance for understanding macroecological processes, general patterns of long-term species occupancy remain largely unknown. We documented the occupancy histories of Cenozoic marine mollusks from New Zealand. For both genera and species, these show a distinct pattern of increase to relatively short-lived peak occupancy at mid-duration, followed by a decline toward extinction. Thus, species at greatest risk for extinction are those that have already been in decline for a substantial period of time. This pattern of protracted rise and fall stands in contrast to that of incumbency, insofar as species show no general tendency to stay near maximal occupancy once established.

Every biologic group, at species rank and higher, varies over time in the size of its footprint on Earth. For example, *Homo sapiens* has spread from a minor species occupying a spot on the globe to complete dominance in many environments, whereas the phylum Brachiopoda has declined in species richness, numerical abundance, and range of occupied environments since the Paleozoic Era. Such observations are part of the basic narrative of the history of life. What is virtually unknown, however, is whether there is any overarching regularity to the waxing and waning of species, although a number of models, with varying predictions, have been proposed on the basis of limited available evidence [summarized in (*1*, *2*)].

Within marine invertebrates over the Phanerozoic Eon, species richness, frequency of occurrence, and geographic range increase and decrease nearly symmetrically on average over the duration of a genus (*3*), although certain subsets of genera show asymmetrical patterns (*3*, *4*). The pattern of regular increase in geographic range has also been demonstrated for Ordovician marine invertebrate genera (*5*). A survey of Late Cretaceous mollusks from the Gulf and Atlantic coasts of the United States found that geographic range and geologic longevity are correlated and that species originating

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shortly before the end-Cretaceous extinction had a statistical distribution of geographic ranges similar to that of species that had originated over a longer span of time leading up to the latest Cretaceous. These observations were used to infer that individual species tend to reach maximal geographic range early in their lifetimes (*6*). It is not known whether the difference between species and genera reflected in these studies holds in general. If so, it could come about because the rise and fall of a genus depends both on the pattern within constituent species and on temporal changes in the number of species within the genus (*3*, *5*).

To examine the history of species and genera, we analyzed Cenozoic marine molluscan data within the Fossil Record File [FRED (www.fred.org.nz/)]. This comprehensive archive of the New Zealand fossil record provides a compilation of taxonomic lists from field-based collections tied to specific localities (*7*). One strength of the FRED data set is that the species in 81% of the collections were identified by just three paleontologists (A. G. Beu, C. A. Fleming, and J. Marwick), often working in collaboration; thus taxonomic consistency is high. The FRED data were downloaded in March 2007 and comprised 6885 collection lists [see supporting online material (SOM)]. From these we eliminated the following: (i) Taxonomic lists for which the identifier of taxa in the collection is unknown or of doubtful reliability. (ii) Collections that could not be assigned to a single New Zealand stage on the basis of their paleontologically or stratigraphically determined age. (iii) Collections from northernmost New Zealand that represent a biogeographically distinct warm-water fauna that is constrained by outcrop to just a brief interval of time (21.7 to 15.9 million years ago) (*8*). (iv) All but level-bottom benthonic taxa that are inferred to have been confined to, or to have ranged into, shelf water depths, because off-shelf environments are inconsistently and poorly sampled [fig. 2 of (*7*)]. Results are similar if the off-shelf and northern data are included (fig. S18). The resulting data were further edited with a variety of automated and manual procedures (*9*) (SOM). After these adjustments, our data set comprised 3974 collections containing 29,361 occurrence records of 2023 species and subspecies and 608 genera and subgenera. Of these species, we conservatively retained 140 extinct, well-sampled species that collectively span 17 stages and about 40 million years (My) (SOM).

Some previous work has found correlations among local abundance, total biogeographic range, and the proportion of that range actually occupied (*3*, *10*–*12*); we therefore include all these concepts under the term "occupancy." Lacking reliable data on numerical abundance, we operationally measured occupancy as the proportion of collections in a given time interval in which a given species occurs. This measure correlates well with geographic range in our data (figs. S5 and S6). Except for rare species in stages with low numbers of collections, this measure, as a simple proportion, is essentially unbiased by the number of collections (figs. S15 and S16). In order to follow the full history of occupancy of each species, we considered only those species that are extinct (SOM). Given that over 98% (138 of 140) of the species we studied are endemic to New Zealand, we have documented nearly the entire history of this set of species.

Occupancy histories can be severely biased by sparsely occurring species, which perforce tend to be at or near maximum occupancy at their times of first and last appearance, but near zero in between (*3*). For this reason, such species cannot yield meaningful occupancy histories. We therefore restricted the analysis to species with a high estimated sampling probability, namely those that occur at least once in every stage within their stratigraphic range, although less stringent culling yields similar results (fig. S10). Given this protocol, and the nature of the fossil record itself, our main analyses strictly apply to the best-sampled species, which are likely to be the most widespread and abundant species and

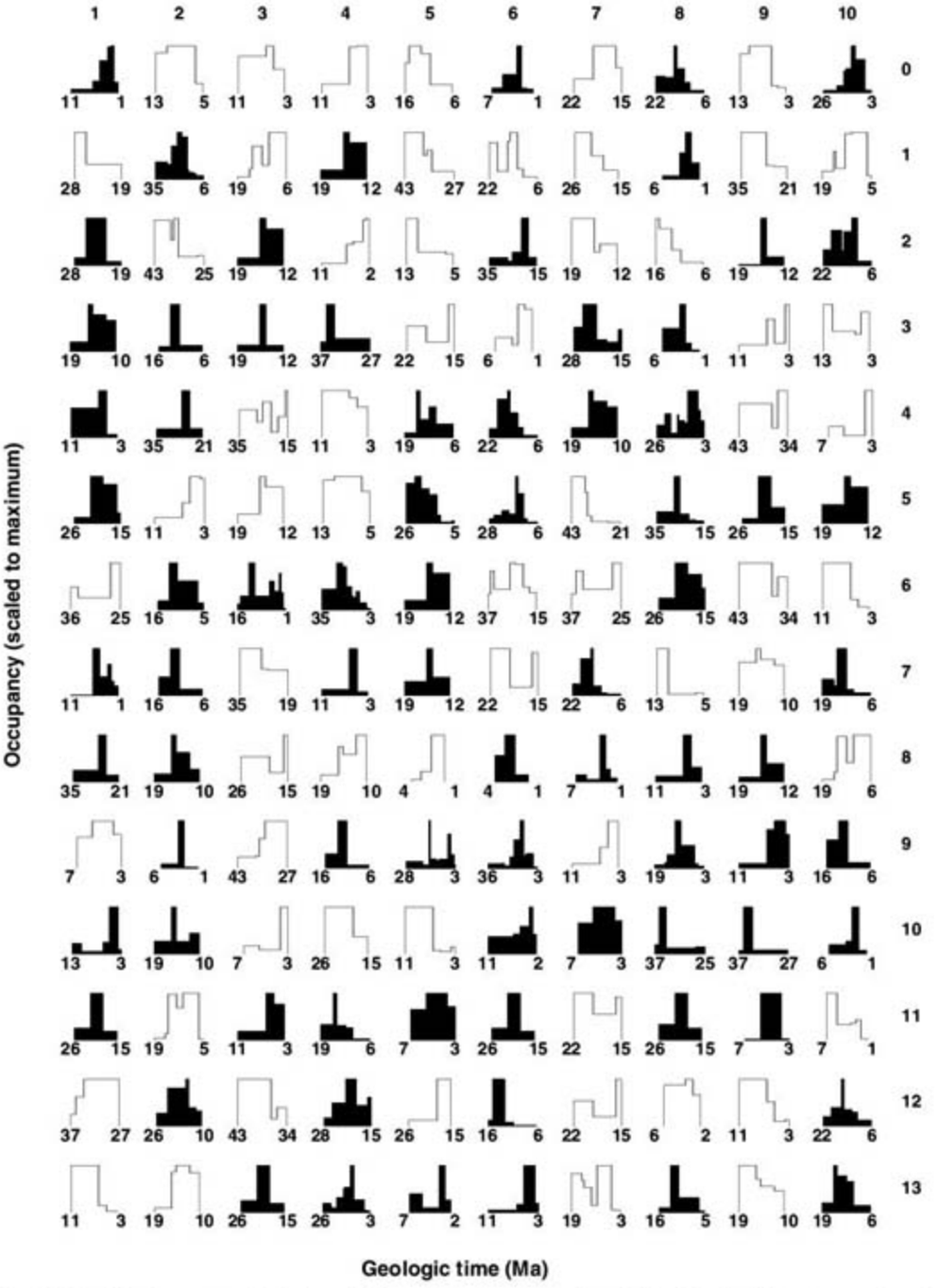

Fig. 1. Empirical occupancy histories of Cenozoic molluscan species of New Zealand. Species are keyed to table S1; for example, species number 16 in table S1 is in row 1, column 6 in this figure. Durations are scaled to unit length from the base of the stage of first appearance to the top of the stage of last appearance, with the given numerical ages in millions of years ago (Ma) based on (*27*). Each occupancy history is scaled to its maximum. Species showing a relatively short-lived peak in occupancy away from the endpoints of the stratigraphic range are shaded.

also the ecological dominants. Large-scale biogeographic patterns are apparently determined disproportionately by common species rather than rare ones (*13*), and we therefore expect our data to capture general trends. We find similar temporal patterns in occupancy and geographic range if we do not cull the data to remove the more sparsely occurring species (SOM).

Because the stratigraphic resolution in our data is at the stage level, no variance in occupancy within stages can be detected and little information on occupancy history can be gleaned from short-lived species. We therefore restricted our analysis to species with a range of three or more stages, although we find compatible results if we include all but single-stage taxa (SOM). In addition to documenting occupancy histories for individual species, we characterized the average occupancy of all species. To aggregate data on species with different durations, we rescaled the stratigraphic range of each species to unit length (*3*). We calculated the height of the average species occupancy history at each point in scaled time as the mean occupancy of all species at that time (SOM). We estimated the uncertainty about this average occupancy history by a bootstrap resampling procedure (SOM). We applied the same protocol to genera.

The occupancy histories for individual species exhibit a variety of shapes (Fig. 1). The most common tendency is for occupancy to increase from the time of first appearance, to attain a relatively short-lived maximum sometime between the stages of first and last appearance, and to decline from this maximum to the time of last appearance (*14*). Roughly 56% of species (78 of 140) exhibit this pattern, a high proportion given that there are several alternatives: 22 species (16%) peak in their stage of first appearance, 20 (14%) peak in their last stage, and 20 (14%) show equivocal patterns. The predominance of species showing the rise-and-fall pattern holds if we consider only those species in which the difference between minimum and maximum occupancy is statistically significant (fig. S11). Statistical analysis of times of peak occupancy shows that these are significantly concentrated toward the middle of species' durations (table S3). There are many individual exceptions and ambiguous cases, but the average species history (Fig. 2) shows that the tendency to reach a peak at mid-duration is a general feature of species-level occupancy. The average occupancy history of species deviates substantially from the relatively constant pattern that would be expected if occurrences within the history of individual species were distributed in proportion to the overall number of occurrences within each stage rather than the relative position of that stage within the species' history (fig. S12). It is possible in principle that the proportion of sites occupied by a species is constant while its local abundance, and therefore its probability of detection, varies over time in a regular way that parallels the occupancy histories we have documented. This would not be an artifact but rather would reflect biologically meaningful variation in an important aspect of occupancy.

Individual and average species occupancy histories in the New Zealand Cenozoic are similar to those for genera (Fig. 2 and fig. S4). Because genera consist of multiple species, the amplitude of the genus curve is higher than that for species. Genera show an earlier peak in average occupancy, but because the number of taxa suitable for our genus-level analysis is small (SOM) and the sampling variance consequently large, this difference in the location of the peak may not be biologically meaningful. Because species are shorter-lived than genera, they rise to maximum occupancy more rapidly in absolute terms, but this rise is nonetheless protracted over several million years (*14*). At their time of extinction, species, like genera, tend to have been in decline rather than being truncated by extinction events at or near their maximum occupancy. As expected given the correlation between occupancy and geographic range (figs. S5 and S6), average histories of geographic range are similar to occupancy histories for New Zealand Cenozoic mollusks (figs. S7 and S8). Our results broadly agree with patterns seen in Cenozoic species of unicellular marine plankton (*15*), a few late Cenozoic mammal species from Italy (*16*), and a larger sample of European Neogene mammal genera (*11*), but they disagree with previous suggestions that species attain their maximal geographic ranges shortly after they originate (*6*).

Because taxa that become extinct at a given point in time are preferentially those that have declined in occupancy and geographic range relative to an earlier peak, our results imply that the prior history of a species is an important determinant of its risk of extinction, and they support the notion that presence over a greater geographic range helps to confer extinction resistance (*17*). Because species tend to take about as long to increase to peak occupancy as to decrease to extinction, our results also suggest that neither the first nor the last appearance can be assumed a priori to be a substantially better biostratigraphic datum in general (*18*).

Easy dispersal is a component of many ecological models that predict substantial shifts in geographic range over short time spans (*19*). In accord with this, rapid range expansions in the present day are well documented (*2*). The contrast with our results suggests that different processes may control occupancy and geographic range on ecological versus geological time scales. Perhaps factors such as dispersal and competitive ability enable species to rapidly fill the ecological and geographic space available to them at any moment, whereas this space in turn is strongly controlled by slowly varying geological processes such as marine transgression and regression (*20*, *21*). It is also likely that many species' ranges are in flux today in response to more rapid anthropogenic forcings and to post-Pleistocene deglaciation and related changes (*22*, *23*).

Finally, ecological incumbency, or the tendency of taxa that already occupy a niche or adaptive zone to resist displacement, is commonly invoked to explain the persistence of certain higher taxa over geological time, even in the face of apparently superior competitors (*24*–*26*). If incumbency were generally important at the species level, we would expect to see most species persisting for long times at or near maximal occupancy. Our findings do not bear this out.

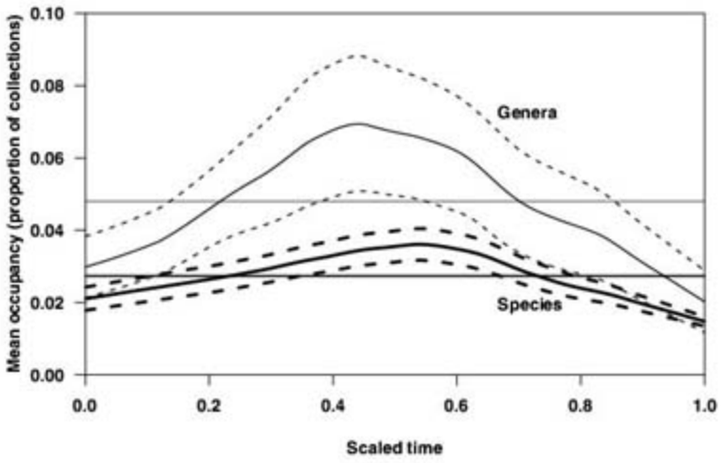

Fig. 2. Average occupancy history of Cenozoic molluscan species (thick curves) and genera (thin curves) of New Zealand, with durations scaled to unit length from the base of the stage of first appearance to the top of the stage of last appearance (*27*). The solid curves are the smoothed averages of the level of occupancy of all taxa at 100 evenly spaced, interpolated points between the times of first and last appearance (SOM). Dashed curves show 1 SE on either side of the average, as estimated from a bootstrap resampling procedure (SOM). The horizontal lines show the overall mean occupancy of all taxa over time.

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14. The geological durations of species in our analysis, from the base of the stage of first appearance to the top of the stage of last appearance, vary from 2.0 to 32 My, with a median [±1 SE (SOM)] of 8.7 ± 0.4 My (*27*). Median durations are 9.3 ± 0.4 My for the shaded species in Fig. 1, and 8.3 ± 0.4 for the remaining species. The median time taken to attain peak occupancy, measured from the base of the stage of first appearance to the midpoint of the peak stage, is 4.0 ± 0.5 My for all species, 3.9 ± 0.5 My for the shaded species in Fig. 1, and 4.6 ± 0.6 My for the remaining species. The median time between the midpoint of the peak stage and the top of the stage of last appearance is 4.5 ± 0.3 My for all species, 4.6 ± 0.4 My for the shaded species in Fig. 1, and 4.5 ± 0.6 My for the remaining species. The subsets of species are statistically indistinguishable from each other in median duration, time from first appearance to peak occupancy, and time from peak occupancy to last appearance (SOM). Nor are there any significant differences between the median time from first appearance to peak and the median time from peak to last appearance (SOM).
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Transgenerational Plasticity Is Adaptive in the Wild

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Plants exhibit adaptive responses to light, but it is not known whether parental plants transmit environmental cues that elicit adaptive responses in offspring. We show that offspring life history (annual versus biennial) is influenced by the maternal light environment (understory versus light gap). This transgenerational plasticity is adaptive when offspring are grown in their maternal light environment, where seeds typically disperse. Projections of population growth show that plants that are appropriately cued for their light environment through maternal effects have 3.4 times greater fitness than otherwise. Transgenerational plasticity has evolved in response to natural variation in light and provides a flexible mechanism by which sedentary organisms cope with heterogeneous environments.

All organisms experience environmental heterogeneity. Some move to cope with variable environments, but those that cannot move require other mechanisms to ensure success. Plants have little choice in their growth environment, and seed dispersal is often limited, with most seeds falling relatively close to the maternal plant (*1*, *2*). Thus, a seedling's growth environment may frequently be similar to its mother's, especially where habitat patches are constant between generations and larger than the scale of seed dispersal. Under these conditions, adaptive maternal cues elicited by the local habitat may evolve if they increase offspring fitness (*3*). In a heterogeneous environment, flexible maternal effects will confer a greater fitness advantage than fixed genetic specialization to local habitats (*4*, *5*), because gene movement between habitats through pollen may result in sampling different environments between generations.

Plants respond to heterogeneity in their immediate environment through plasticity. This plasticity may be adaptive, enhancing individual performance (*6*), or simply a passive consequence of resource limitation (*7*, *8*). Plasticity may also occur between generations if the parental environment influences the expression of offspring traits. Transgenerational plasticity in response to maternal environments is common in plants (*9*–*12*), and maternal effects may increase maternal fitness (*10*). There is little evidence that transgenerational plasticity enhances offspring fitness in plants, although conditions, such as sedentary growth form, spatially patchy habitats, and limited seed dispersal, favor its evolution (*10*, *13*). The demonstration of adaptive maternal effects requires that (i) the maternal environment influences offspring trait expression, (ii) these maternal influences are genetically based, and (iii) maternal effects enhance offspring fitness.

Campanulastrum americanum is a monocarpic herb whose populations span distinct light environments and include annual and biennial life histories. Seeds that germinate in the fall are annuals, whereas those germinating in the spring are biennials and flower during their second summer. Annual and biennial plants cooccur, and an individual's seeds may germinate in either or both seasons. We hypothesized that adaptive maternal effects may influence the life-history schedule, because germination time de-

Table 1. Analysis with generalized linear models of the influence of offspring life history and of maternal and offspring light environment on fitness components in *C. americanum*. For seedling yield, life history indicates the probability of germination and early survival as annuals and biennials. Dashes indicate factors not included in the model. *, $P < 0.10$; †, $P < 0.05$; ‡, $P < 0.01$; §, $P < 0.001$.

	Seedling yield (χ^2)	Rosette survival (χ^2)	Adult survival (χ^2)	Fruit number (F)	Seeds per fruit (F)
Life history (LH)	13.14§	-	9.54‡	41.13§	14.55§
Offspring light (OL)	10.40‡	0.00	0.58	39.09§	16.89§
Maternal light (ML)	2.86*	0.00	0.07	0.41	0.31
OL × ML	8.86‡	1.98	0.00	0.23	0.06
OL × LH	947.80§	-	15.43§	0.16	0.78
ML × LH	7.61‡	-	0.29	0.63	0.40
OL × ML × LH	0.02	-	0.11	0.15	0.00
Block	845.26§	19.69	167.08§	2.50‡	1.70†
Herbivory	-	-	-	9.02§	0.80

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termines life history and maternal effects are usually strongest in seeds (*9*, *10*). To evaluate possible adaptive maternal effects, we asked (i) whether maternal light environment influences offspring life history, (ii) if there is genetic variation for maternal light effects, and (iii) whether these maternal effects increase offspring fitness.

Our central hypothesis was that offspring fitness would be enhanced when plants were grown in the same light environment as that of the maternal plant. *C. americanum* individuals either grow in the forest understory, which receives no direct sunlight, or in tree-fall light gaps and are in full sunlight for part of each day. On average, light gaps receive 10 times the irradiance as understory habitats (*14*) (fig. S1). Plasticity to light is relatively well understood at the phenotypic and molecular level (*15*), and plastic shade-avoidance responses are often adaptive (*16*, *17*). Light gaps are large relative to the scale of *C. americanum*'s seed dispersal (*13*), therefore offspring typically grow in the same light environment as that of their mother. It is less likely that offspring share their father's light environment, because *C. americanum* outcrosses (*18*) and is pollinated by bumblebees (*19*) that forage over areas encompassing both understory and light-gap habitats.

We pollinated full-sib mothers in the two environments with the same sire to create genetically similar offspring that differed in their maternal light environment. We planted seeds in light-gap and understory areas of the population with a partial reciprocal transplant experimental design (*14*). Both maternal and offspring light environments influenced the number of annuals and biennials that germinated and survived as seedlings (seedling yield). Most seeds in light gaps germinated in the fall as annuals, and most seeds in the understory germinated in the spring as biennials (offspring light; Table 1 and Fig. 1). Seedling yield was also determined by the maternal light environment (offspring light × maternal light; Table 1). Seeds in light gaps had greater germination rates and early survival if their mother was from a light gap for both annuals (offspring light × maternal light; $F = 7.14$, $P < 0.008$) and biennials (maternal light; $F = 4.42$, $P < 0.04$; Fig. 1). Seeds in the understory had greater germination and survival if their mother was from the understory, but this was true only for annuals (Fig. 1). Maternal effects influenced offspring life-history schedule and enhanced offspring germination and survival when seeds were grown in the same light environment as that of their mother for three of the four combinations.

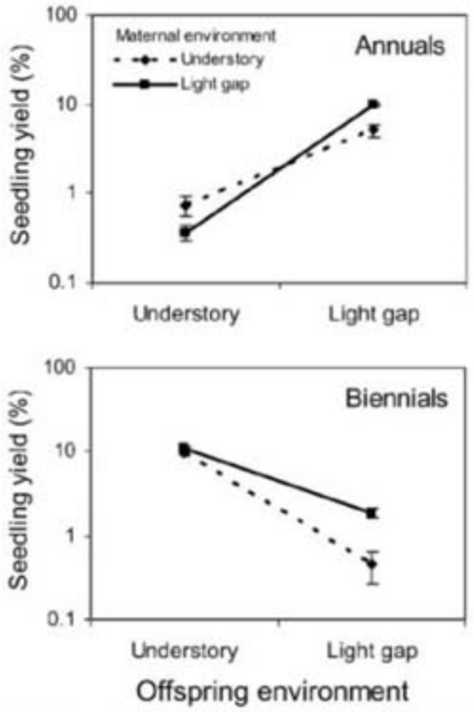

Fig. 1. The influence of maternal and offspring light on the number of annual (fall-germinating) and biennial (spring-germinating) *C. americanum* seedlings. Maternal and offspring plants were grown in forest understory or light-gap habitats. Seedling yield (± SEM) is the percentage of seeds that germinated and survived through the seedling stage of the life cycle.

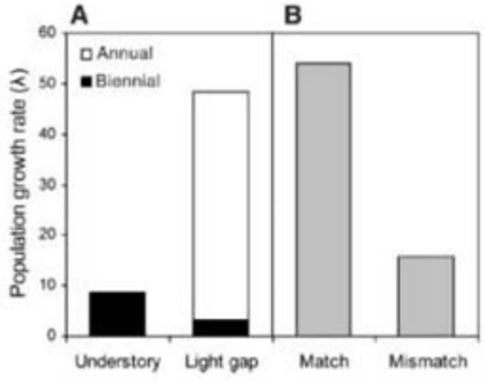

Fig. 2. (**A**) The contribution of annual and biennial life histories to projected population growth in *C. americanum* in forest understory and light-gap habitats. (**B**) Projected population growth when offspring are grown in their maternal light environment (match) or when the light environment is switched between generations (mismatch). [Natural populations are expected to have growth rates (λ) of near unity; larger growth rates are a product of experimental conditions favoring germination and survival.]

We evaluated the performance of annuals (fall germinating) and biennials (spring germinating) in understory and light-gap habitats by manipulating life history such that annuals and biennials bloomed in the same year (*14*). Seeds were obtained from maternal plants that were reared in understory and light-gap environments. These seeds were germinated under controlled conditions in the spring so that they expressed a biennial life history. Seeds germinating in the fall were used as annuals. Biennial and annual seedlings were transplanted in subsequent years into light-gap and understory areas of the natural population. Maternal light environment did not directly influence adult fitness traits (Tables 1 and 2). In contrast, offspring growth environment affected almost all fitness components. Adult survival was greater for annuals in light gaps and for biennials in the understory (offspring light × life history; Tables 1 and 2), mirroring seedling yield. Plants in light-gap habitats had at least 5.5 times greater fruit production and 1.5 times as many seeds per fruit as understory plants (Tables 1 and 2).

We compared the fitness of annual and biennial plants in each light environment using a population projection approach. In most monocarpic plants, fitness is measured as fecundity, with individuals that do not survive to reproduce scored as zero. However, in *C. americanum*, population growth rate (λ), estimated by projection matrix analysis, is a more appropriate measure than fecundity because of the differences in life span between annuals and biennials. The contribution of annual and biennial life histories to population growth was determined with demographic loop analysis (*14*, *20*), where the loop elasticity indicates the proportion of population growth contributed by each life history. 93.5% of the projected population growth in light gaps is due to annuals (Fig. 2A), despite the greater fruit and seed production of biennials (Tables 1 and 2). In contrast, annuals growing in the forest understory contribute only 0.3% to population, growth. Overall, projected population growth is larger in light gaps than in the understory (Fig. 2A). Greater frequency and fitness of biennials in light-limited regions of the population, and annuals in light gaps where resources are more abundant, support predictions of life-history theory (*21*, *22*) and suggest that seasonal germination plasticity, in response to the ambient light environment, enhances fitness. Furthermore, the differential performance of annuals and biennials between light habitats indicates that environmental heterogeneity helps maintain the polymorphic life history in this species.

Table 2. Mean (SEM) rosette survival, adult survival, fruit number, and number of seeds per fruit for *C. americanum* growing in light-gap and understory habitats. N.A., not applicable.

	Annual		Biennial	
	Light gap	Understory	Light gap	Understory
Rosette survival (%)	N.A.	N.A.	96.9	97.4
Adult survival (%)	69.8	50.0	63.0	83.3
Fruit number	38.1 (2.5)	3.1 (0.3)	192.9 (9.6)	35.0 (1.0)
Seeds per fruit	25.5 (1.1)	8.1 (1.4)	38.1 (1.0)	25.4 (0.6)

To determine the adaptive value of maternal light effects, we analyzed λ in plants with both matching and mismatched maternal and offspring light environments. Frequency and fitness were greater for annuals in light gaps and biennials in the understory. The projection matrix mimicked these conditions with data for annuals from seeds of sunlight-grown mothers planted in light gaps and for biennials from seeds of shade-grown mothers planted in the understory (*14*). The conditions of a second projection matrix were the same as those of the first, except that offspring were not grown in their mother's environment. A demographic loop analysis was conducted on each matrix. λ for mismatched maternal and offspring environments was less than a third of that when they matched (Fig. 2B), indicating that maternal environmental effects enhance offspring fitness.

We tested whether maternal light effects were genetically variable, focusing on germination of light-gap annuals and understory biennials from families with all combinations of maternal and offspring environments. The effect of the maternal light environment on the proportion of fall-germinating seeds in light gaps and spring-germinating seeds in the understory varied among families (life history × family × maternal light; $\chi^2 = 37.20$, df = 1, $P < 0.011$; table S1), indicating genetic variation for maternal environment effects on seedling traits that influence fitness.

We demonstrated that offspring have greater rates of germination and early life survival when planted into their maternal environment and that these early life effects influence fitness as measured by λ. Because seed dispersal is limited, most *C. americanum* individuals will experience their maternal light environment. Maternal effects that enhance performance in this environment represent transgenerational adaptive plasticity and allow for phenotypic adaptation to local environmental conditions. In plants, which lack the ability to escape their environment, maternal effects may serve as a form of environmental cuing between generations that enhance offspring performance. Maternal environmental effects should be favored over fixed specialization when genes move between environments via pollen and occasionally via seed. Seeds that disperse outside the maternal environment will suffer reduced fitness for a generation, but recovery is expected in locally produced offspring. As such, maternal effects represent a flexible evolutionary mechanism for sedentary organisms to cope with heterogeneous environments.

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Widespread Monoallelic Expression on Human Autosomes

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Monoallelic expression with random choice between the maternal and paternal alleles defines an unusual class of genes comprising X-inactivated genes and a few autosomal gene families. Using a genome-wide approach, we assessed allele-specific transcription of about 4000 human genes in clonal cell lines and found that more than 300 were subject to random monoallelic expression. For a majority of monoallelic genes, we also observed some clonal lines displaying biallelic expression. Clonal cell lines reflect an independent choice to express the maternal, the paternal, or both alleles for each of these genes. This can lead to differences in expressed protein sequence and to differences in levels of gene expression. Unexpectedly widespread monoallelic expression suggests a mechanism that generates diversity in individual cells and their clonal descendants.

In diploid eukaryotic organisms, it is generally assumed that the maternally and paternally derived copies of each gene are simultaneously expressed at comparable levels. However, there are exceptions where only one of the alleles is expressed. Monoallelically expressed genes fall into three distinct classes. One class is the autosomal imprinted genes (such as *IGF2* and *H19*) whose monoallelic expression is regulated in a parent-of-origin–specific manner (*1*). A second class is X-inactivated genes regulated by a random process: Early in development, around the time of implantation, half of the cells inactivate the maternal X chromosome and half inactivate the paternal X chromosome (*2*). A third class is autosomal genes subject to random monoallelic expression (*3–9*), including the odorant receptor genes, as well as genes encoding the immunoglobulins, T cell receptors, interleukins, and natural killer cell receptors. For genes in this class, some cells express the maternal allele and other cells express the paternal allele. For some genes, cells expressing both alleles are also present [e.g., members of the interleukin gene family (*5*, *6*)]. This third class was considered to comprise isolated examples of genes involved in the immune or nervous systems. Here, we present the development of a method for genome-wide identification of such genes, which revealed that more than 5% of assessed genes were subject to random monoallelic expression.

To carry out a genome-wide search for genes subject to random monoallelic expression, we used the Affymetrix Human Mapping 500 K array set, modifying the protocol to allow examination of RNA rather than DNA (*10*) (fig. S1). The locations of single-nucleotide polymorphisms (SNPs) on the Affymetrix 500 K SNP array are arbitrary with respect to locations within genes, with ~1% falling within exonic sequence and ~36% within intronic sequence. To allow the use of intronic SNPs, we purified nuclei so as to enrich intronic RNA. This RNA was then converted into double-stranded cDNA and used

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in place of genomic DNA in an Affymetrix genotyping protocol (figs. S1 to S4 and table S1). The search also took advantage of the observation that once a cell has decided to express one of two alleles (both in the case of X-inactivation and in the examples of autosomal monoallelic expression), the clonal descendants of this cell maintain the choice (*2*, *5*, *6*, *8*, *9*, *11*). Because normal human B-lymphoblastoid cell lines are polyclonal, we derived clonal cell lines using single-cell cloning.

The overall approach thus generated "transcriptome-derived genotypes," which we could compare to the regular genotype obtained from genomic DNA from the same clonal cell line. A homozygous transcriptome-derived genotype in the context of the same SNP yielding a heterozygous genomic DNA-derived genotype served to identify monoallelic expression. We developed an algorithm that used genotyping calls from independent replicate hybridizations of cDNA from each clonal cell line. To be conservative, we applied a number of filters to the data (*12*). These filters discarded potentially interesting observations (imprinting and allelic imbalance due to cis regulatory sequence polymorphism) but were essential to avoid possible cDNA genotyping artifacts. Overall, ~10% of SNPs were reliably called from cDNA; this was expected, because most of the other SNPs are likely present in regions of the genome with insufficient transcription in the B cell lines we analyzed (*13*).

In a proof-of-principle analysis, our approach detected the chromosome-wide nature of X-inactivation in female clonal cell lines [including its subtle properties (*14*–*16* and supporting online text)] while detecting biallelic expression in nonclonal cells (Fig. 1). The X chromosome is represented by 5710 SNPs on the Nsp 250 K array. Analyzing individual H, 1294 of 5710 X-linked SNPs were heterozygous, and 135 of these heterozygous SNPs were called in cDNA from two or more clonal lines (*17*). X-inactivation was also observed in clonal cell lines from a second female (fig. S5). The majority (88 of 135) of these X-linked SNPs were within known annotated genes, whereas the rest were in areas currently labeled as intergenic regions. Nonetheless, these intergenic SNPs correctly report X-inactivation. Although allele-specific reverse transcription polymerase chain reaction (RT-PCR) experiments confirmed monoallelic expression for intergenic SNPs both on the X chromosome and on the autosomes, we focus here on the SNPs that were within genes.

Having tested the cDNA genotyping approach on the X chromosome, we set out to systematically search for autosomal genes displaying random monoallelic expression. Examination of all the SNPs within a gene allowed an assignment of allele-specific expression to each gene in each clone (Fig. 1B and fig. S6). Across the entire genome, for the genes whose transcription we could assess, an average of 2.75 informative SNPs were called per gene. The overall agreement between SNPs present within a single gene was >98% (based on analyses of >10,000 instances in clones from each of three people). The agreement among SNPs present in a given gene extended to both exonic SNPs and intronic SNPs (fig. S5).

For a given gene subject to monoallelic expression, there are three possible types of expressing clones: monoallelic-paternal, monoallelic-maternal, and (for some genes) clones expressing both alleles. The *APP* (amyloid precursor protein) gene serves as an example of how we interpreted the cDNA genotyping data in general. Additionally, *APP* is important because of its involvement in Alzheimer's disease. Analyses of cDNA from clones of individual H revealed monoallelic expression of *APP* (Fig. 2, A and B); some clonal cell lines displayed monoallelic expression (either the maternal or the paternal allele), whereas another clonal cell line revealed biallelic expression. Individual A also displayed clear evidence of monoallelic expression of the *APP* gene, whereas individual M had only two clonal cell lines, each of which displayed biallelic expression. Although this might imply that in individual M the *APP* gene is expressed exclusively biallelically, it could also be due to the small number of clones we were able to analyze for this individual. Direct sequencing of RT-PCR products, and genotyping of RT-PCR products using primer-extension, both served to confirm monoallelic expression of *APP* (Fig. 2C). These experiments also showed that the active allele has at least 50 times as much expression as the silent allele (*10*). SNPs reporting monoallelic expression of *APP* were located in multiple introns along most of the length of the gene (Fig. 2D).

How is the level of expression of a given gene affected by its transcription from one allele or both? One possibility is that the transcriptional activity of each allele is regulated independently, such that having both alleles active leads to higher overall transcript level. The alternative possibility is that the cell maintains a desired level of transcript, whether one or two alleles are transcribed. When assessed by quantitative real-time RT-PCR, clones expressing *APP* from one allele, either paternal or maternal, had a lower level of expression than the biallelic clones (Fig. 2E). In the case of *APP*, this is especially pertinent because higher levels of expression and *APP* gene duplication are associated with early-onset Alzheimer's disease (*18*). Therefore, by contributing to diversity in levels of expression in individual cells and their clonal descendants, monoallelic expression could play a role in pathogenesis.

Another example of a gene displaying monoallelic expression is the early B cell factor (*EBF*) gene. Although lymphoblasts are relatively easy to subclone and thus are a natural subject for our analysis, we were also able to analyze a clone from the WI38 primary fibroblast line. This clone also revealed monoallelic expression of *EBF* and a number of other genes (Fig. 2F, fig. S7, and table S5). Analyses of *EBF* also included a control experiment showing that, as expected, the mature mRNA and the unspliced RNA display monoallelic expression of the same allele (Fig. 2G and fig. S8).

To verify our results in vivo, we used RNA fluorescence in situ hybridization (RNA-FISH) to detect nascent transcripts in nuclei of monocytes from fresh peripheral blood mononuclear cells (PBMCs). DNA-FISH allowed the localization of the two alleles. We analyzed the *EBF* gene as well as the death-associated protein kinase 1 (*DAPK1*) gene (Fig. 2H and fig. S9). For both of these genes, about one-third of the cells revealed monoallelic transcription, consistent with the results from clonal cell lines, indicating that a fraction of clones display monoallelic expression.

Another in vivo experiment analyzed small (1 mm^3) patches of tissue from a female placenta.

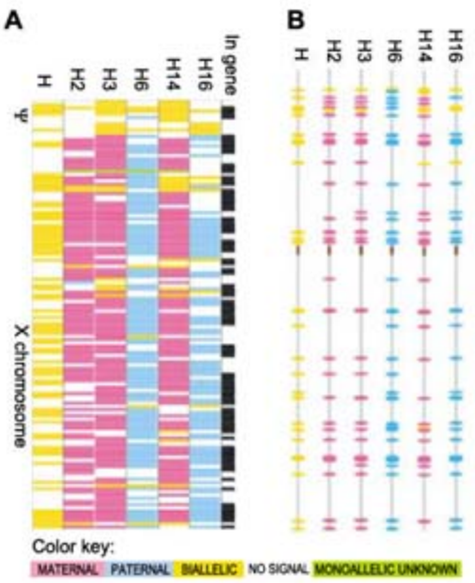

Fig. 1. Proof-of-principle: detection of X-inactivation. (**A**) SNP-level view of chromosome-wide X-inactivation in individual clonal lines. Each column corresponds to cDNA genotypes from one cell line from the same female; each row, to one SNP. Only informative SNPs are shown; top to bottom, in the order of ascending coordinate on the X chromosome [National Center for Biotechnology Information (NCBI) 35 assembly]. SNPs located within known genes are marked black in the rightmost column; the rest are in the intergenic regions. Ψ denotes pseudoautosomal region. H is the line prior to subcloning. (**B**) Gene-level view of X-inactivation in individual clonal lines. Each oval represents a gene. The active allele was assigned based on complete agreement of all informative SNPs within the gene. The center of the oval corresponds to position of the gene on the NCBI 35 assembly, and all ovals are of equal size. Brown boxes denote centromeres.

Analyses of large samples showed the expected biallelic expression of X-linked and autosomal genes. Patches displaying complete skewing of X-inactivation (fig. S10) were tentatively considered "clonal," and RNA from these patches was analyzed with the Affymetrix 500 K SNP platform. Figure 2I shows examples of genes displaying monoallelic expression in small patches of placental tissue. The data obtained from these in vivo "clones" were qualitatively similar to the data from the B lymphoblast clones (fig. S10 and table S6). These data provide another independent, in vivo confirmation of random monoallelic expression.

Having presented a few autosomal examples and control experiments, we now turn to a genome-wide view of monoallelic expression (Fig. 3A). On the Nsp 250 K array, there are SNPs present within 11,401 genes. Overall, we were able to assess allele-specific transcription for 3939 genes in two or more clonal cell lines; to be assessable, a given gene had to have at least one heterozygous SNP that gave a genotype call from cDNA. We devised a metric that assigns a score to each gene, reflecting the extent to which the data support the conclusion that the gene is monoallelically expressed (*10*).

Of the 3939 genes, 2.2% (85) were called as monoallelically expressed with multiple informative SNPs per gene per clone; these genes were grouped together as class I. Eight arbitrarily selected genes from class I were checked by

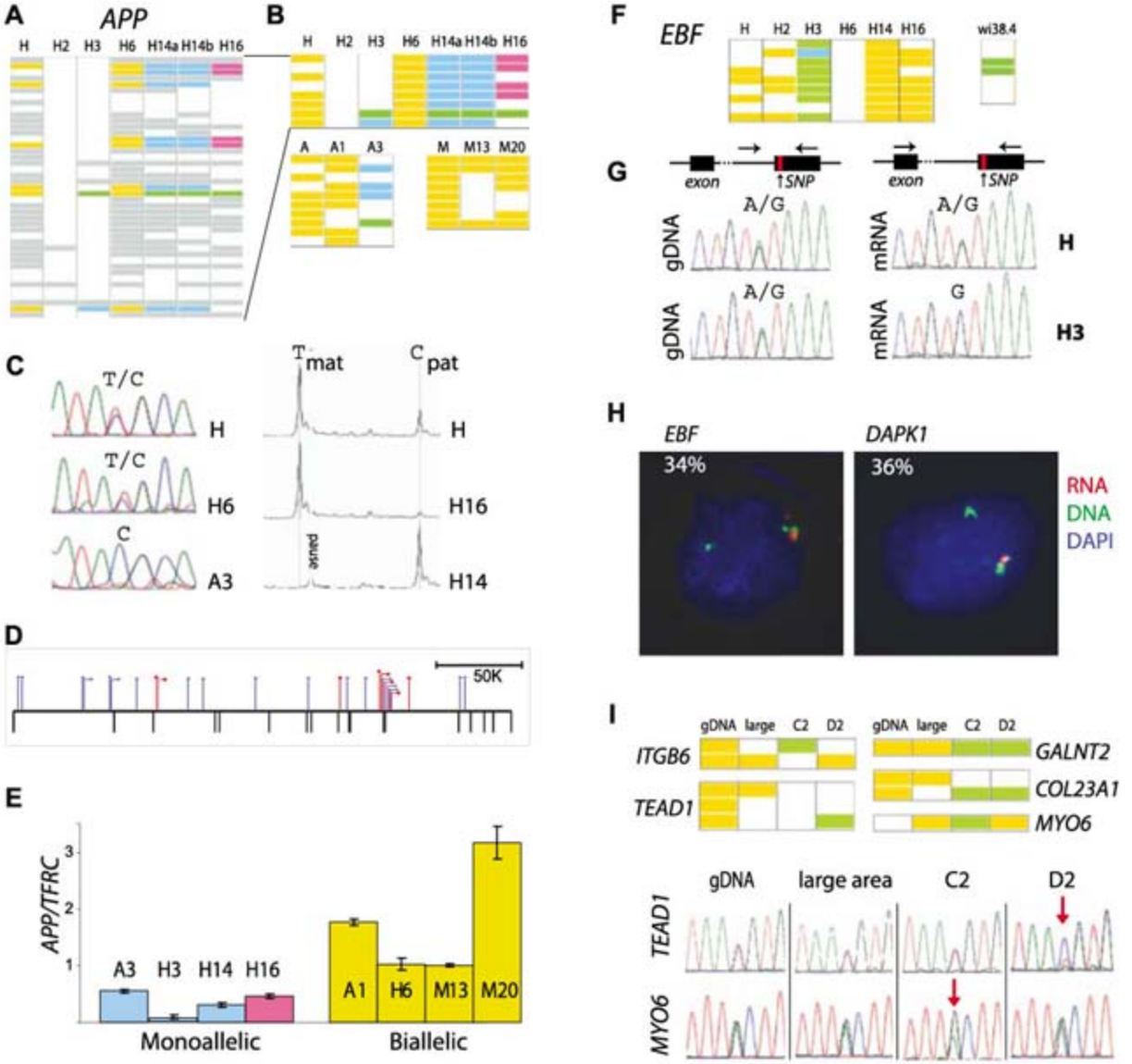

Fig. 2. Monoallelic expression in autosomal genes. (**A**) Monoallelic expression of APP. Columns, uncloned cell line (H) or one of the independent clones; rows, SNPs, identified by color as in Fig. 1; gray, homozygous genomic DNA. (**B**) Shows only the SNPs heterozygous in genomic DNA and with cDNA calls. Data from two additional individuals (A and M) are included. (**C**) Sequencing of RT-PCR products surrounding an intronic SNP, RS1783026 (left). Mass spectrograms of single-base extension reactions on PCR products surrounding the same SNP (Sequenom, right). "Pause" denotes the position of a peak distinct from either allele. (**D**) Positions of assessed SNPs within APP; homozygous (blue) or heterozygous (red) in individual H. Exons demarcated below the line in black. (**E**) Relative levels of APP expression in monoallelic and biallelic clonal lines as measured by real-time RT-PCR, normalized to *TFRC* transcription; an average of triplicate measurement ± SD. (**F**) A clone derived from primary lung fibroblasts (WI38.4) also showed monoallelic expression of the *EBF* gene. (See also fig. S7.) Parental genotypes for WI38 line were not available. (**G**) Confirmation of monoallelic expression in mature *EBF* mRNA, using intron-spanning primers for amplification; SNP RS1368298 assessed. (**H**) Monoallelic expression of *EBF* and *DAPK1* in fresh PBMCs. Sequential in situ hybridization with probes for nascent RNA (red) and DNA (green) shows a large fraction of nuclei with transcription from only one allele ($n = 50$). Nuclei counterstained with 4′,6′-diamidino-2-phenylindol (blue). (**I**) Mosaic of monoallelic expression in fresh tissue. Small (~1 mm^3) samples, dissected from female placenta, were essentially clonal judging by X chromosome methylation assay (fig. S10). Examples of monoallelic (in lymphoblasts) genes assessed by Nsp 250 K array in placenta are shown. Direct sequencing of cDNA flanking RS1440284 (*TEAD1*) and RS2208798 (*MYO6*) confirmed allelic bias in expression (arrows), compared with gDNA and cDNA prepared from a larger sample of the same tissue.

Sequenom genotyping and were confirmed (table S2). An additional 7.3% of assessed genes (286) were called as monoallelically expressed based on a single informative SNP per gene per clone, or their score was reduced for other reasons described in (*10*); these we group together as class II. Of six genes from class II checked by Sequenom genotyping, five were confirmed (table S2). The genes from class I and II include both B cell–specific genes and ubiquitously expressed genes expressed at typical levels in B cells (Table 1). Although extrapolating to the entire transcriptome is complicated because the arrays we used are biased toward larger genes (as they have more SNPs; see fig. S11), conservative interpretation of our data still suggests that more than 1000 human genes are subject to random monoallelic expression.

For greater than four-fifths of the monoallelic genes, some clonal cell lines displayed biallelic expression. These observations are consistent with prior studies of other monoallelic genes [for example, interleukins 2 and 4 (*6*, *8*), as well as p120 catenin (*9*)]. Thus, the genes that display only monoallelic expression, such as odorant receptors and immunoglobulins, are the exception rather than the rule.

The genes subject to monoallelic expression were scattered throughout the genome (Fig. 3B). Within a given clonal cell line, the choice of the expressed allele (maternal, paternal, or both) was made independently for each gene (Fig. 3C). This is in contrast to the chromosome-wide coordination observed in X-inactivation; it is also interesting given the chromosome-wide coordination of asynchronous replication observed for autosomes (*19*, *20*). Chromosome-wide coordination of asynchronous replication timing therefore does not lead to chromosome-wide coordination of random monoallelic expression. Thus, individual clones displayed epigenetic heterogeneity, in terms of which autosomal genes were monoallelic and which were biallelic (as well as the parent-of-origin of the expressed allele in the cases of monoallelic expression). Given the large number of genes involved, numerous combinations are potentially generated by monoallelic expression (fig. S12).

The newly identified monoallelically expressed genes encode proteins of widely varying functions and tissue specificities, and some of them have been studied for roles in human disease (Table 1; see also supporting online text). This diversity notwithstanding, a disproportionately large fraction of these monoallelically expressed genes encode cell surface proteins (table S3). For example, examining the Gene Ontology category "transmembrane receptor" using GOstat (http://gostat.wehi.edu.au) (*21*), the expected number of monoallelically expressed genes would be 8 (3.0%), although 24 (8.8%) were observed ($P = 2 \times 10^{-6}$ after Benjamini correction for multiple hypothesis testing). The overrepresentation of receptors and other surface proteins suggests a role for monoallelic expression in each given cell's interactions with other nearby cells.

The evolutionary history and species specificity of random monoallelic expression remain to be determined. It is thus intriguing that the genes we identified are more than twice as likely as biallelic genes to be located near presumed regulatory conserved noncoding sequences that were recently suggested to undergo human lineage-specific accelerated evolution (*22*) ($P < 10^{-6}$; fig. S13). This observation suggests the possibility of human-lineage–specific random monoallelic expression.

Using a general strategy for genome-wide analysis of monoallelic expression, we showed that ~5 to 10% of assessed autosomal genes display random monoallelic transcription in human cells with stability in each clonal cell line. Corroborating data were obtained from in vivo experiments examining fresh individual blood cells with RNA-FISH and from allele-specific RT-PCR analyses of small patches of apparently clonally derived tissue from the placenta. RNA-FISH analyses of clonal cell lines biallelic for a given gene revealed biallelic expression in individual cells ruling out a rapid switching mechanism. Further evidence for stability comes from our previous demonstration in mouse clonal cell lines that the choice of a single active allele is highly stable and that biallelic clonal cell lines give rise only to biallelic subclones (*9*). A number of unusual regulatory mechanisms have been observed for monoallelically expressed genes (*20*, *23*, *24*), the most famous of which is immunoglobulin gene DNA rearrangement (*25*). For the immunoglobulin gene and few other monoallelically expressed genes, allele-specific DNA methylation has been observed (*26*, *27*). Given the heterogeneity in the new genes we have identified, diverse mechanisms likely impact their allele-specific expression. Conservative extrapolation from our data suggests that at least 1000 autosomal human genes are subject to random monoallelic expression. This monoallelic expression can impact on biological function by creating three distinct cell states for each given gene when the two alleles encode functionally different proteins. These states (for each given gene) would be defined by expression of the maternal allele, the paternal allele, or both alleles. Monoallelic expression can also contribute to cellular (or clonal) diversity even without polymorphism, as demonstrated by our observation of higher expression levels in clones expressing both alleles as compared with clones with monoallelic expression. Previous examples wherein monoallelic expression has been shown to be

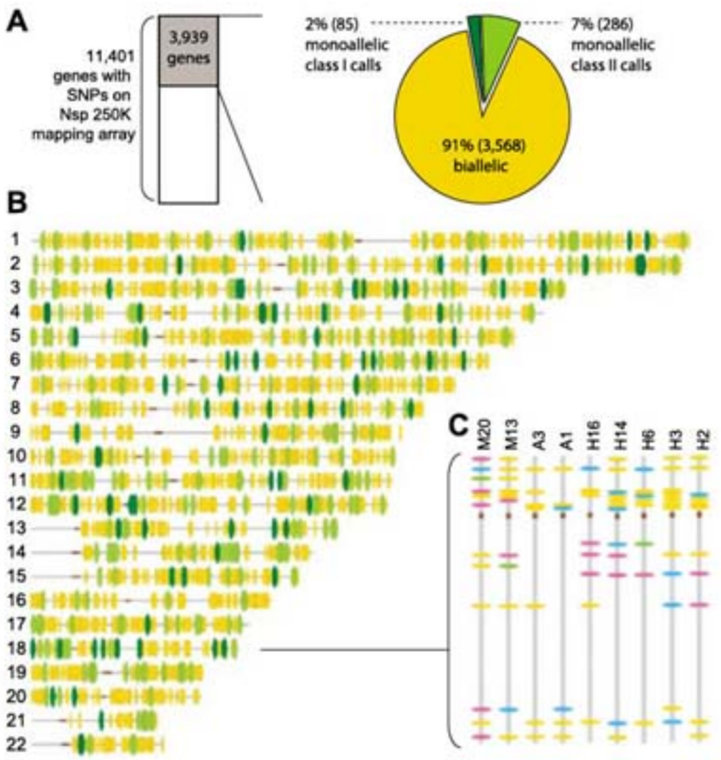

Fig. 3. Widespread monoallelic expression on the human autosomes. (**A**) Of 11,401 genes, 3939 were assessable; a metric *G* (*10*) assigned a monoallelic quality score for each gene for each individual. Genes with high ($G_{max} > 1$, dark green) or medium ($G_{max} = 1$, light green) scores are shown and are included in further analyses. Genes with only biallelic expressing clones are in gold. The complete list of assessed genes is in database S1. (**B**) Distribution along autosomes of randomly monoallelically expressed and biallelic genes we identified. Each marker corresponds to the position of the gene on the NCBI 35 assembly. Brown boxes mark centromeres. (**C**) Gene-level view of allele-specific expression on chromosome 18 in clonal cell lines from three individuals.

Table 1. Partial list of genes subject to random monoallelic expression. G_{max} is the highest score for the metric G among the three assessed individuals. A measure of expression level in lymphoblasts is given in Sanger expression: Average raw expression level for lymphoblasts in HapMap set (*28*). Tissue specific: Ratio of the level of expression of the highest expressing tissue to the median expression for all tissues in GNF Gene Expression Atlas (*29*). Using the averages for all probes representing a given gene, gene expression was determined to be tissue-specific if the ratio was >4. B cell specific: Ratio of the expression (averaged over all probes for the gene and over all replicate samples) in peripheral blood CD19+ B cells and B721 cells to the median expression of the gene in all tissues. See also table S4 and database S1.

Gene	Monoallelic assignment class	G_{max}	Chr	Sanger expression	Tissue specific	B cell specific	Description
ABR	II	1.00	17	1270	4.0	1.27	Active BCR-related gene
APP	I	2.88	21	388	3.0	0.42	Amyloid beta (A4) precursor protein
COCH	II	1.00	14	661	10.7	2.63	Coagulation factor C homolog, cochlin (*Limulus polyphemus*)
COMT	I	2.70	22	1132	5.9	1.05	Catechol-O-methyltransferase
CRY1	II	1.00	12	169	2.9	0.80	Cryptochrome 1 (photolyase-like)
CXCL10	II	1.00	4	383	4.7	0.64	Chemokine (C-X-C motif) ligand 10
CYP7B1	I	9.00	8	302	2.3	1.10	Cytochrome P450, family 7, subfamily B, polypeptide 1
DPP4	II	1.00	2	125	14.5	0.57	Dipeptidylpeptidase 4 (CD26)
EBF	I	18.00	5	518	5.0	2.82	Early B cell factor
EPHB1	I	2.78	3	138	4.8	0.81	EPH receptor B1
EPS8	II	1.00	12	582	5.5	0.65	Epidermal growth factor receptor pathway substrate 8
EVC	I	9.00	4	464	4.2	0.87	Ellis van Creveld syndrome
FAH	II	1.00	15	701	12.0	0.92	Fumarylacetoacetate hydrolase (fumarylacetoacetase)
GHR	I	1.50	5	137	9.1	5.53	Growth hormone receptor
LMO2	II	1.00	11	451	5.4	0.80	LIM domain only 2 (rhombotin-like 1)
ME1	I	25.00	6	707	7.6	1.49	Malic enzyme 1, NADP(+)-dependent, cytosolic
PLD1	II	1.00	3	172	5.6	2.28	Phospholipase D1, phophatidylcholine-specific
PREP	II	1.00	6	558	2.8	1.98	Prolyl endopeptidase
RALBP1	II	1.00	18	737	4.7	1.96	ralA binding protein 1
ROBO1	I	7.00	3	570	9.9	5.21	Roundabout, axon guidance receptor, homolog 1 (*Drosophila*)
TCL1A	II	1.00	14	8235	22.0	12.15	T cell leukemia/lymphoma 1A
TIRAP	II	1.00	11	143	3.5	1.17	Toll-interleukin 1 receptor (TIR) domain containing adaptor protein
UST	II	1.00	6	238	3.9	0.84	Uronyl-2-sulfotransferase
ZNFN1A1	I	2.50	7	532	5.3	2.45	Zinc finger protein, subfamily 1A, 1 (Ikaros)

essential for the proper functioning include the immune system's antigen receptors (*4*) and the olfactory system's odorant receptors (*7*). The stability of the allele-specific choice in a given clone taken together with clonal expansion to form tissues can lead to macroscopic patches of tissue with subtly different properties, as we have observed in analyses of placental tissue. For any given tissue, the size of patches will be dependent on the time in development that allelic choice is made. Thus, in the brain and other tissues, each individual would be predicted to be a mosaic with respect to allele-specific expression of numerous autosomal genes, providing an epigenetic basis for functional differences amongst individuals.

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12. We limited our analyses to SNPs that were heterozygous in genomic DNA (from a given individual) and that were consistent with Mendelian inheritance of the parental genotypes. As an additional control, we verified heterozygosity in genomic DNA from each clone. When analyzing cDNA genotypes, we eliminated all SNPs for which we consistently detected only one of the two alleles (or for which only one allele was detected in the nonclonal cell line). A further filter was to consider only SNPs that gave a cDNA genotype call for at least two distinct clones.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5853/1136/DC1
Materials and Methods
SOM Text
Figs. S1 to S13
Tables S1 to S6
Database S1

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Promotion of Tissue Inflammation by the Immune Receptor Tim-3 Expressed on Innate Immune Cells

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$CD4^+$ T helper 1 (T_H1) cells are important mediators of inflammation and are regulated by numerous pathways, including the negative immune receptor Tim-3. We found that Tim-3 is constitutively expressed on cells of the innate immune system in both mice and humans, and that it can synergize with Toll-like receptors. Moreover, an antibody agonist of Tim-3 acted as an adjuvant during induced immune responses, and Tim-3 ligation induced distinct signaling events in T cells and dendritic cells; the latter finding could explain the apparent divergent functions of Tim-3 in these cell types. Thus, by virtue of differential expression on innate versus adaptive immune cells, Tim-3 can either promote or terminate T_H1 immunity and may be able to influence a range of inflammatory conditions.

Inflammatory responses are regulated through multiple pathways that often involve subsets of $CD4^+$ T helper cells, and much effort has been devoted to understanding the key pathways that regulate these cells. Tim-3 was identified as a member of the TIM (T cell immunoglobulin and mucin domain) family, specifically expressed on $CD4^+$ T_H1 and not T_H2 lymphocytes (*1*). We have shown that interactions of Tim-3 and its ligand play a role in regulating T_H1 responses as well as contributing to T cell tolerance (*2*, *3*). More recently, galectin-9 was identified as a Tim-3 ligand that could dampen T_H1 immunity by inducing cell death in effector T_H1 cells (*4*). Moreover, TIM-3 expression is low in T cell clones isolated from the cerebrospinal fluid of patients with multiple sclerosis (MS) (*5*). Collectively, these data support a key role for Tim-3 in regulating T_H1 responses and human autoimmune diseases.

In addition to its expression in T cells, *Tim-3* mRNA is present in $CD11b^+$ cells (*1*) and other non-T cells, although the functional relevance of this is not known (*6*–*8*). Further analysis here revealed that Tim-3 was specifically restricted to $CD11b^+$ dendritic cells (DCs) and not $CD11b^+$ macrophages (Fig. 1A). Analysis of monocytes and DCs from peripheral blood of healthy human subjects also revealed that human DCs expressed high levels of TIM-3, although it was also detected at low levels in human monocytes (Fig. 1B).

We next tested the production of cytokines from wild-type and Tim-$3^{-/-}$ DCs in response to lipopolysaccharide (LPS) as a positive control, or galectin-9 plus LPS. Although a small level of tumor necrosis factor–α (TNF-α) was secreted in response to LPS stimulation alone, this was much greater in combination with galectin-9 and considerably diminished in Tim$3^{-/-}$ DCs (Fig. 1C).

To more specifically study the effects of Tim-3 triggering in DCs, we generated an antibody agonist of Tim-3 (anti–Tim-3) (*9*). Because we observed that DCs produce TNF-α ex vivo in response to Tim-3 ligation, we stimulated the dendritic cell line D2SC1 with anti–Tim-3. Consistent with the previous cytokine expression data, engagement of Tim-3 led to a specific induction of nuclear factor κB (NF-κB) (Fig. 1D), confirming the induction of an inflammatory transcription factor cascade. Using a blocking anti–TIM-3, we observed 75% inhibition of galectin-9–mediated TNF-α secretion from human monocytes (Fig. 1E). Collectively, these data show that Tim-3 is highly expressed by cells of the innate immune system in both mice and humans, and that its expression on antigen-presenting cells promotes the secretion of proinflammatory cytokines from monocytes and dendritic cells.

Peripheral bone marrow–derived monocytes give rise to microglia in the central nervous system (CNS) (*10*, *11*), and both resident microglia and infiltrating monocytes have been shown to contribute to CNS inflammation (*12*). To explore possible TIM-3 expression on monocyte cells in the CNS, we first examined its expression in autopsy tissue from subjects with no evident inflammatory disease (*9*). TIM-3 staining was apparent in white but not gray matter parenchyma on cells with a histological appearance consistent with microglia (Fig. 2A). Staining with CD11b confirmed the expression of TIM-3 on microglia in CNS white matter (Fig. 2B). We

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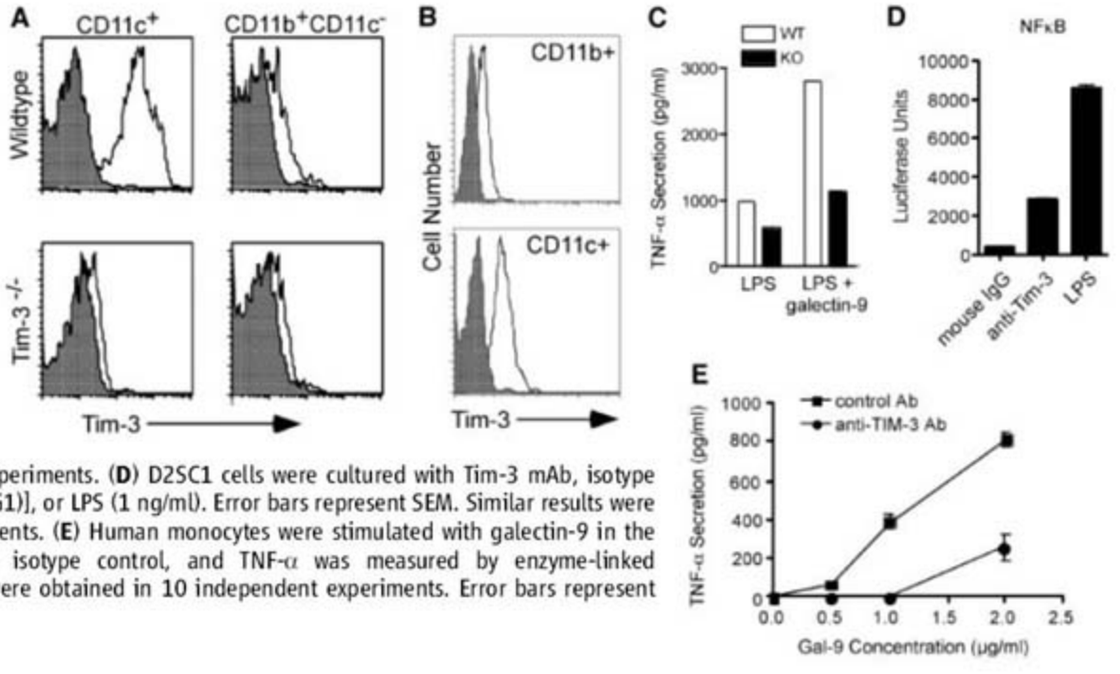

Fig. 1. Tim-3 expression and function in innate cells. (**A** and **B**) Spleen cells from wild-type and Tim-$3^{-/-}$ mice (A) and peripheral blood mononuclear cells from a healthy human subject (B) were stained with monoclonal antibodies (mAbs) to CD11b, CD11c, and Tim-3 or relevant isotype controls. Open histograms, Tim-3; shaded histograms, isotype control. (**C**) Splenic DCs from wild-type or Tim-$3^{-/-}$ mice were isolated and cultured with LPS, LPS + galectin-9, or medium. Cytokine production in culture supernatant was measured by cytometric bead array. Similar results were obtained in three independent experiments. (**D**) D2SC1 cells were cultured with Tim-3 mAb, isotype control [mouse immunoglobulin G1 (IgG1)], or LPS (1 ng/ml). Error bars represent SEM. Similar results were obtained in three independent experiments. (**E**) Human monocytes were stimulated with galectin-9 in the presence of blocking TIM-3 mAb or isotype control, and TNF-α was measured by enzyme-linked immunosorbent assay. Similar results were obtained in 10 independent experiments. Error bars represent SD in cytokine.

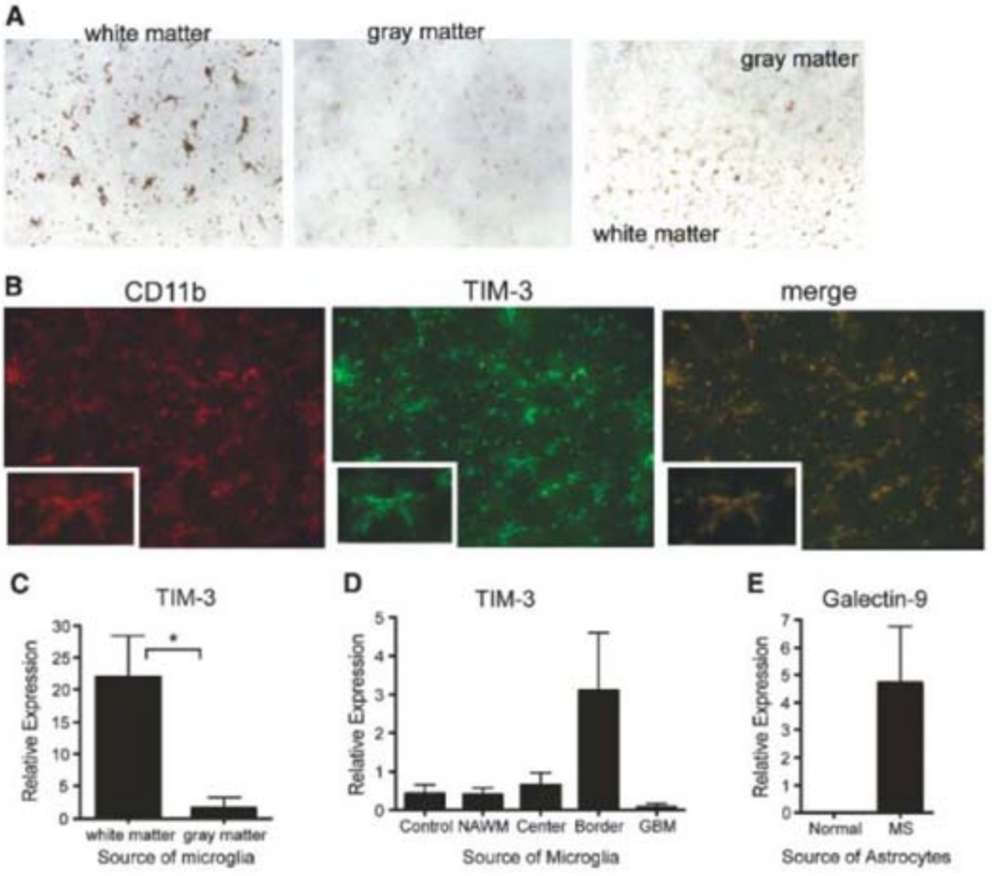

Fig. 2. TIM-3 expression in human microglia. (**A**) Tissue sections from white and gray matter regions of human noninflamed CNS tissue were stained with TIM-3–specific mAb (magnification, 20×). (**B**) Dual immunofluorescence of noninflamed CNS white matter tissue using CD11b and TIM-3 mAbs (magnification, 20×; insets, 40×). (**C**) Quantitative RT-PCR analysis of *TIM-3* mRNA levels in microglia isolated using LCM from white and gray matter regions of CNS tissue. Error bars represent SD in *TIM-3* mRNA levels among five experiments. Gray matter microglia express significantly lower levels of TIM-3 ($P = 0.02$, two-tailed t test). (**D**) Microglia were isolated from noninflamed (control) human CNS tissue ($n = 2$), normal-appearing white matter (NAWM) regions of MS tissue ($n = 2$), the center ($n = 4$) or border ($n = 3$) regions of active MS plaques, or glioblastoma multiforme (GBM) tumor specimens ($n = 3$), and levels of TIM-3 were determined by quantitative RT-PCR. Error bars represent SEM. (**E**) Astrocytes were isolated by LCM from noninflamed white matter ($n = 2$) and from MS plaques ($n = 5$) from two different brain specimens. Galectin-9 expression was determined by RT-PCR. Error bars represent SEM.

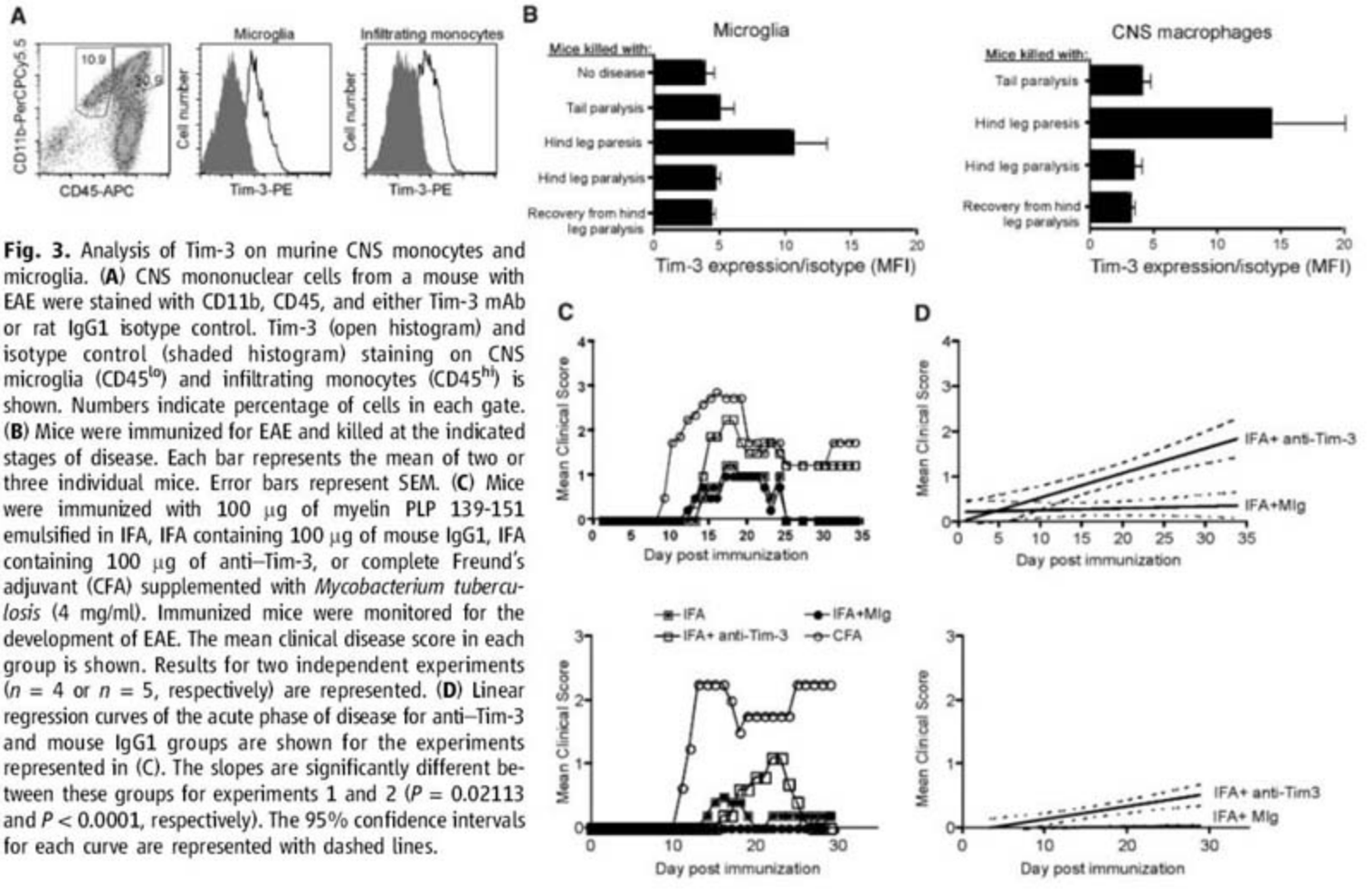

Fig. 3. Analysis of Tim-3 on murine CNS monocytes and microglia. (**A**) CNS mononuclear cells from a mouse with EAE were stained with CD11b, CD45, and either Tim-3 mAb or rat IgG1 isotype control. Tim-3 (open histogram) and isotype control (shaded histogram) staining on CNS microglia ($CD45^{lo}$) and infiltrating monocytes ($CD45^{hi}$) is shown. Numbers indicate percentage of cells in each gate. (**B**) Mice were immunized for EAE and killed at the indicated stages of disease. Each bar represents the mean of two or three individual mice. Error bars represent SEM. (**C**) Mice were immunized with 100 μg of myelin PLP 139-151 emulsified in IFA, IFA containing 100 μg of mouse IgG1, IFA containing 100 μg of anti–Tim-3, or complete Freund's adjuvant (CFA) supplemented with *Mycobacterium tuberculosis* (4 mg/ml). Immunized mice were monitored for the development of EAE. The mean clinical disease score in each group is shown. Results for two independent experiments ($n = 4$ or $n = 5$, respectively) are represented. (**D**) Linear regression curves of the acute phase of disease for anti–Tim-3 and mouse IgG1 groups are shown for the experiments represented in (C). The slopes are significantly different between these groups for experiments 1 and 2 ($P = 0.02113$ and $P < 0.0001$, respectively). The 95% confidence intervals for each curve are represented with dashed lines.

then used laser capture microdissection (LCM) to isolate CD11b$^+$ cells from normal white and gray matter tissues for quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis (*9*). In these analyses, little or no *TIM-3* mRNA could be detected in microglia obtained from gray matter tissue, whereas it was clearly present on microglia from white matter tissue (Fig. 2C).

To test the possible consequences of TIM-3 in the CNS, we examined TIM-3 expression on infiltrating monocytes and microglia isolated from the white matter CNS tissue of patients with MS and from CNS glioblastoma multiforme (GBM) tumors. Although lymphocytes as well as monocytes and microglia are associated with these pathologies, the cytokine profiles differ considerably (*13*, *14*). Thus, the T_H1 cytokines interferon-γ (IFN-γ) and TNF-α associate with MS but not with GBM tissues. Monocytes and microglia captured from the active border regions of MS lesions expressed higher levels of TIM-3 than did those captured from the quiescent center of MS lesions, from adjacent normal-appearing white matter, or from noninflamed white matter tissue (Fig. 2D). In contrast, TIM-3 expression was significantly lower in monocytes and microglia obtained from GBM tissues, relative to those obtained from control tissue or MS tissue.

Although galectin-9 is undetectable on resting human astrocytes, it can be up-regulated by the cytokines interleukin-1 and IFN-γ (*15*–*17*), leading us to examine whether it might be induced on astrocytes present in MS lesions. Indeed, galectin-9 levels were significantly elevated on astrocytes present in MS lesions relative to normal human CNS tissue (Fig. 2E). Collectively, these data reveal that both TIM-3 and its ligand are up-regulated on microglia and astrocytes, respectively, in inflamed white matter tissue.

To further explore the mechanism underlying selective TIM-3 expression on white matter microglia, we examined Tim-3 expression on peripheral CD11b$^+$ macrophages in mice immunized for the induction of experimental autoimmune encephalomyelitis (EAE), a murine model of MS (*18*). In these animals, activated peripheral CD11b$^+$ cells failed to express Tim-3, although CD11b$^+$ monocytes infiltrating the CNS from the periphery, distinguished from resident microglia by higher expression of CD45 (*19*, *20*), did express Tim-3 (Fig. 3A and fig. S1). Moreover, levels of Tim-3 on both microglia and infiltrating monocytes peaked just before the peak of clinical disease (Fig. 3B).

The earlier experiments showing high Tim-3 on DCs and TNF-α secretion are consistent with the notion that triggering Tim-3 on DCs promotes inflammatory T_H1 responses. To examine this further, we induced EAE by immunizing mice with myelin proteolipid protein (PLP) 139-151, emulsified in incomplete Freund's adjuvant (IFA) containing either anti–Tim-3 or an isotype control. We observed that immunization in the presence of anti–Tim-3 led to disease with greater severity (Fig. 3C), which also showed a statistically significant difference in disease course.

To address whether Tim-3 engagement induces distinct signaling in innate and adaptive immune cells, we stimulated a Tim-3–expressing CD4$^+$ T cell clone and a DC cell line with anti–Tim-3 and examined them for tyrosine phosphorylation. Differences were observed in the proximal signaling pathways triggered by Tim-3 in T cells and DCs (Fig. 4A). Specifically, tyrosine phosphorylation was induced in two molecules after Tim-3 engagement in T cells but not in DCs, and phosphorylation of a third molecule was induced in DCs but not in T cells. In contrast, engagement of Tim-3 led to similar degrees of extracellular signal–regulated kinase activation and degradation of the NF-κB inhibitor IκBα in the two cell types (Fig. 4, B and C). Although the magnitude of ERK phosphorylation induced by Tim-3 appeared to be lower in the DCs, phosphorylation induced with a positive control stimulus [phorbol 12-myristate 13-acetate (PMA)] was also lower in these cells.

Our results show that Tim-3 serves opposing roles in the innate and adaptive immune systems. In the naïve resting immune system, Tim-3 promotes inflammation. This is consistent with our data indicating that Tim-3 is primarily expressed on DCs in the naïve state and that Tim-3 synergizes with Toll-like receptors to promote TNF-α secretion. Once T_H1 responses are generated, Tim-3 is expressed on terminally differentiated T_H1 cells, which will outnumber DCs and induce the up-regulation of galectin-9 via their production of IFN-γ (*15*, *16*). Finally, galectin-9 triggers Tim-3 on T_H1 cells to terminate T_H1 immunity. In sum, our data show how a single molecule, Tim-3, by virtue of differential expression on cells of the innate and adaptive immune systems, can both promote inflammation and terminate T_H1 immunity. Thus, Tim-3 may represent a valid therapeutic target in a wide range of peripheral and organ-specific human inflammatory diseases.

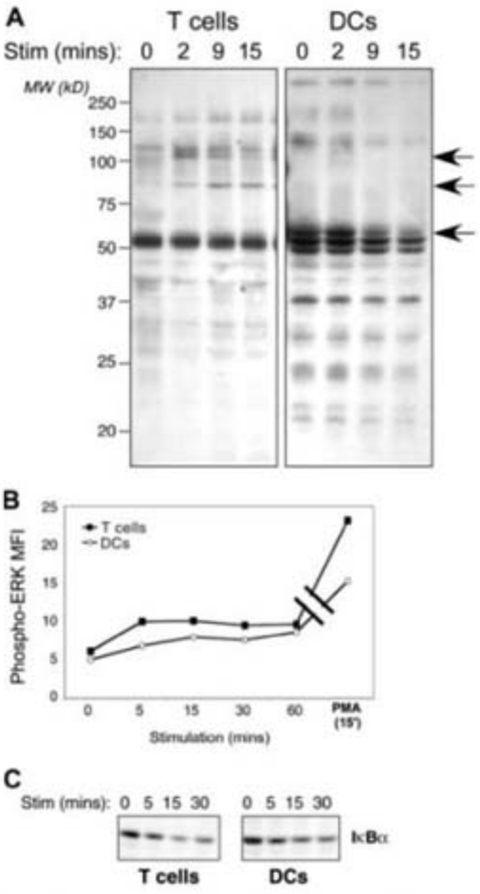

Fig. 4. Differential Tim-3 signaling in T cells versus DCs. T cells (T_H1 clone AE.7) or DCs (D2SC1) were stimulated for the indicated times with anti–Tim-3. (**A**) Cells were lysed with NP-40 lysis buffer and analyzed by SDS–polyacrylamide gel electrophoresis (PAGE) and Western blotting with phosphotyrosine mAb 4G10 (Upstate/Chemicon). The top two arrows indicate the position of tyrosine-phosphorylated substrates uniquely induced in T cells; the bottom arrow indicates a substrate uniquely induced in DCs. (**B**) Cells were fixed with paraformaldehyde and permeabilized with cold methanol for intracellular staining with a mAb to phospho-ERK (BD Biosciences). As a control, a sample of each cell type was also stimulated with PMA. (**C**) Cells were lysed with NP-40 lysis buffer and analyzed by SDS-PAGE and Western blotting for total IκBα (Cell Signaling Inc.). Data in (A), (B), and (C) are representative of three independent experiments.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5853/1141/DC1
Materials and Methods
Fig. S1

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Melatonin Suppresses Nighttime Memory Formation in Zebrafish

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Memory processes are modulated by the biological clock, although the mechanisms are unknown. Here, we report that in the diurnal zebrafish both learning and memory formation of an operant conditioning paradigm occur better during the day than during the night. Melatonin treatment during the day mimics the nighttime suppression of memory formation. Training in constant light improves nighttime memory formation while reducing endogenous melatonin concentrations. Treatment with melatonin receptor antagonists at night dramatically improves memory. Pinealectomy also significantly improves nighttime memory formation. We adduce that melatonin is both sufficient and necessary for poor memory formation during the night.

A circadian influence on learning and memory has been shown in both diurnal and nocturnal invertebrates (*1*–*3*) as well as nocturnal vertebrates (*4*). The mechanism of this circadian influence on memory is not known. In our study, we used zebrafish (*Danio rerio*) to investigate whether the circadian system plays a regulatory role in learning and memory formation in a diurnal vertebrate. An active-avoidance conditioning (AAC) paradigm in which the fish were placed within a training tank with two compartments was used. The fish were trained to associate a compartment within the tank having a light signal (conditioned stimulus) as the safe environment and a dark compartment associated with mild electric shocks (unconditioned stimulus) as the unsafe environment (*5*). We first asked whether zebrafish show diurnal differences in acquisition and long-term memory (LTM) for AAC. Animals entrained to 14 hours light:10 hours dark (LD) cycles were trained to learning criteria at two time points (ZT8 and ZT16) and then tested 24 hours later. Animals trained during the day reached criteria significantly faster than animals trained at night (Fig. 1A). Furthermore, zebrafish trained and tested during the day had significantly better long-term (24 hours) retention (LTR) for AAC than animals trained during the night (Fig. 1B). The retention score reflects the ratio between training and testing performances using the algorithm shown in (*5*).

To determine whether the endogenous circadian system controls this rhythm, we examined AAC performance in constant conditions. Animals previously entrained to LD cycles were exposed to constant darkness (DD). On the third and fourth day of DD, the animals were trained and tested at different times (Fig. 1, C and D). Similar to LD, zebrafish in DD show significant phase-dependent differences in both acquisition and LTR. Animals trained during the subjective day (SD) in DD and tested 24 hours later reached learning criteria faster and had significantly better LTR compared with the animals that were trained and tested during the subjective night (SN; Fig. 1, C and D).

Because animals learned the task during the night but showed no LTR, probably either the formation or the retrieval of the newly acquired memory is being modulated. To differentiate these possibilities, we trained and tested two groups of animals in opposite circadian phases such that group 1 was trained on the third day of DD at relative Zeitgeber time 8 (rZT8) (SD) and tested 36 hours later at rZT20 (SN), whereas group 2 was trained at rZT16 (SN) and tested 36 hours later at rZT4 (SD). Acquisition of AAC was again shown to be better during the SD (group 1) than during the SN (group 2) (Fig. 1E). When both groups were tested 36 hours later, group 1 had a significantly higher retention score than group 2 (Fig. 1F). Thus, the observed rhythm in 24-hour memory (Fig. 1D) is primarily due to a circadian modulation of LTM formation rather than memory retrieval.

We next asked whether the endogenous circadian system modulated the formation of multiple forms of memory. The decay of AAC memory was measured for animals trained on the third day of DD at rZT8 (SD) or at rZT16 (SN). Animals tested 30 min after training showed no significant difference in short-term AAC performance whether they were trained during SD or SN (Fig. 1G; $P > 0.05$). However, animals trained at rZT8 had significantly higher 1-hour retention scores than animals trained at rZT16 (Fig. 1G). By 3 hours after training, the retention scores for both groups of animals had reached the same amount (Fig. 1G). The rapid decay of short-term retention after acquisition during the SN coincided with the absence of LTM for AAC.

We hypothesized that melatonin is responsible for inhibiting memory formation during the night. In zebrafish, the rhythm of melatonin release from the pineal gland is modulated by both its endogenous circadian oscillator and also directly by light (*6*). Melatonin concentrations are high during the night and lower during the day. The rhythm in LTM for animals kept in 14:10-hour LD cycles (Fig. 1B) was much more robust than the rhythm observed in DD (Fig. 1D), consistent with light enhancing the rhythm. In other vertebrates, melatonin can modulate synaptic function, which may affect memory formation (*7*–*9*).

If melatonin modulates LTM formation, then we would expect a change in the amplitude of LTM of AAC by modulating physiological melatonin amounts. To test for sufficiency, we applied melatonin before training during the SD. This treatment had no effect on acquisition of AAC across all concentrations tested (fig. S1A). Furthermore, animals treated with either 1 μM or 10 μM of melatonin before training did not show a significant effect on LTM as compared to the control (Fig. 2A). However, at 50 μM or 100 μM of melatonin, LTM formation was significantly suppressed (Fig. 2A). Interestingly, animals treated with 50 μM melatonin directly after training did not show a significant effect on LTM formation (fig. S2A), which suggests that the inhibitory action of melatonin on LTM formation occurs at a relatively early stage of LTM formation. In fact, melatonin pretreatment also produces a substantial decay in early memory, much more severe than normally seen after daytime acquisition but similar to that seen after nighttime acquisition (compare fig. S2A and Fig. 1G).

If melatonin was responsible for the poor LTM of AAC during the night, then melatonin treatment should affect memory formation and not retrieval. Thus, animals trained at rZT8 (SD) were treated with 50 μM melatonin for 1 hour before testing. The effect of melatonin present during testing on LTM of AAC was not significantly different from the vehicle presentation (fig. S2B). This result further indicates that melatonin largely does not inhibit animal performance during AAC. Moreover, this result is consistent with the hypothesis that endogenous melatonin has a modulatory role during LTM formation because both night and melatonin affect memory formation and not recall.

The ability of melatonin to mimic AAC memory formation during the SN suggested that signaling through endogenous melatonin receptors inhibits memory formation. Therefore, we examined whether melatonin receptor antagonists would block the effect of melatonin on AAC memory formation during the SD. Animals were treated on the third day of DD for 1 hour with vehicle, melatonin, or melatonin in the presence of a melatonin receptor antagonist [luzindole or K-185 (*10*)]. Both melatonin receptor antagonists effectively blocked the inhibitory effect of melatonin on LTM formation (Fig. 2B). Thus, the effect of melatonin on LTM is mediated through the activation of melatonin receptor signaling pathway(s).

The melatonin hypothesis also predicts that reducing melatonin signaling should improve memory formation during the SN. Animals were treated for 1 hour before training with the melatonin receptor antagonist luzindole or vehicle during the SN. After treatment, animals were trained for AAC and tested 24 hours later. There were no significant differences in the time to reach learning criterion between animals treated

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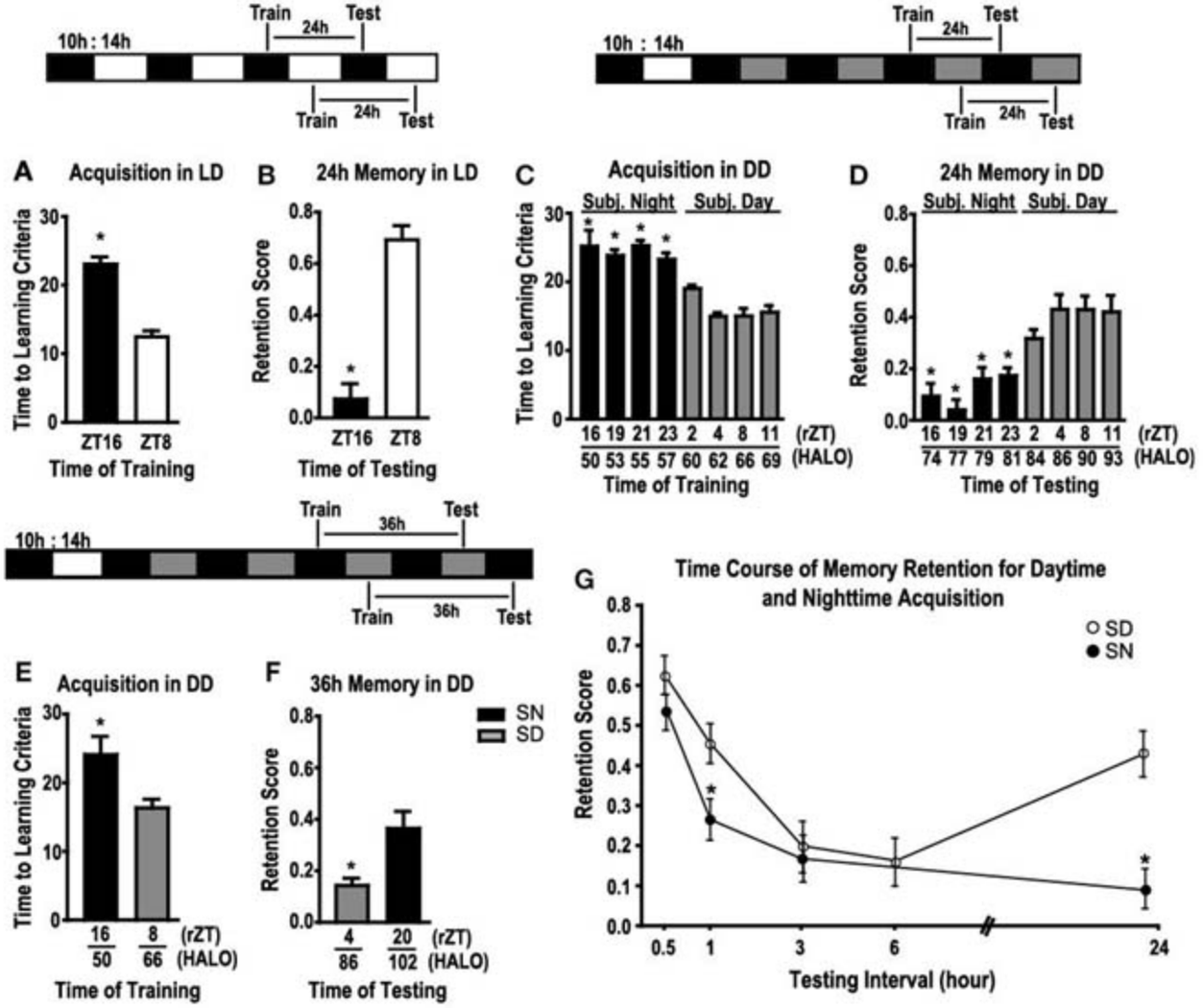

Fig. 1. Phase differences in memory acquisition and LTM formation. (**A**) Animals showed significantly shorter training periods to learning criteria at ZT8 compared with animals trained at ZT16 ($t = 6.432$, $P < 0.0001$) (*5*). (**B**) Diurnal rhythm in LTR. Animals tested 24 hours after training at ZT8 showed significantly better retentions for AAC compared with animals that were originally trained at ZT16 ($t = 7.429$, $P < 0.0001$). (**C**) Circadian modulation of acquisition. Fish that were trained during the SN required significantly longer to learn compared with animals trained during the SD [$F_{(7,49)} = 11.14$, $P < 0.0001$] (*5*). (**D**) When animals trained during SD were tested 24 hours later, retentions for the acquired task were significantly better than those from animals that were trained during SN [$F_{(7,49)} = 10.43$, $P < 0.0001$]. (**E** and **F**) Circadian modulation of LTM formation and not memory retrieval. (**E**) Animals trained at rZT8 ($n = 6$) showed a significant reduction in the training period compared with animals trained at rZT16 ($n = 7$) ($t = 2.64$, $P < 0.05$). (**F**) Animals trained at rZT8 and tested 36 hours later showed significantly increased LTR for AAC compared with animals trained at rZT16 and tested 36 hours later ($t = 6.26$, $P < 0.005$). (**G**) Time course of memory retention. Animals were tested at specific time intervals after training at rZT8 and rZT16 (*5*). The results show a circadian modulation of LTR and 1-hour retention for AAC [$F_{(7,48)} = 12.43$, $P < 0.005$].

with luzindole and the control (fig. S1C), indicating that the melatonin antagonist did not influence acquisition during the SN, as was the case for melatonin treatment alone during the SD (fig. S1A). In contrast, blocking melatonin signaling with 5 μM luzindole during the SN significantly improved 30-min, 1-hour, and 24-hour memory for AAC (Fig. 3, A and B). Similar results were found for K-185 (fig. S3).

During constant light exposure, zebrafish maintain rhythmic locomotor activity (*11*), but melatonin concentrations are constitutively low, even during SN (*12*, *13*). Interestingly, acquisition was not significantly different for groups subjected to either LL or DD, suggesting that endogenous melatonin concentrations do not have a major effect on learning AAC (fig. S1D). However, consistent with the melatonin hypothesis of memory modulation, animals subjected to LL showed a significant and robust improvement in LTM during the SN as compared to animals subjected to DD (Fig. 3C).

The pineal gland is the principal source of melatonin in cold-blooded vertebrates (*14*, *15*). Animals that underwent pinealectomy along with sham-operated animals were trained during both the SD (rZT8) and the SN (rZT16) on the third day of DD and tested for LTM 24 hours later. There were no significant differences between the pinealectomized and sham-operated animals in either acquisition (fig. S1E) or LTM (Fig. 3D) during the SD. Acquisition of AAC during the SN for the pinealectomized and sham-operated animals also was not different (fig. S1F). However, during the SN, the pinealectomized animals displayed significantly better LTR than sham-operated animals (Fig. 3E).

Memory formation of AAC is decreased during the SN relative to the SD. Our data also argue that the poor nighttime memory formation occurs through an active repression induced by melatonin. Melatonin can inhibit memory for-

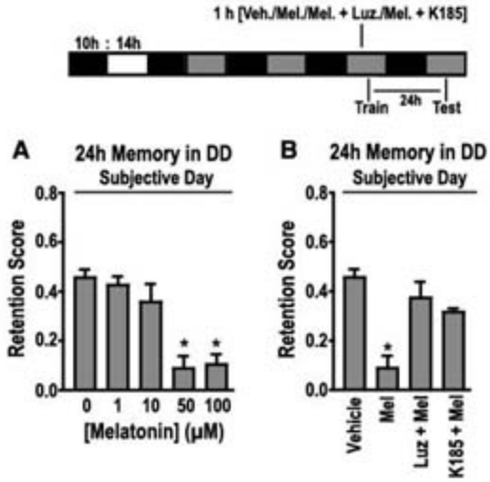

Fig. 2. Melatonin is sufficient for poor nighttime LTM. (**A**) Animals previously trained in the presence of melatonin (*5*) showed a concentration-dependent effect of melatonin on LTM formation [$F_{(4,41)} = 12.51$, $P < 0.001$; Newmann-Keuls posthoc analysis for control versus 50 μM and 100 μM, $P < 0.001$, and for control versus 1 μM and 10 μM, $P > 0.05$]. (**B**) Animals trained for AAC after treatment with melatonin and either luzindole or K-185 showed normal SD time LTR [$F_{(2,19)} = 1.5$, $P > 0.26$] (*5*).

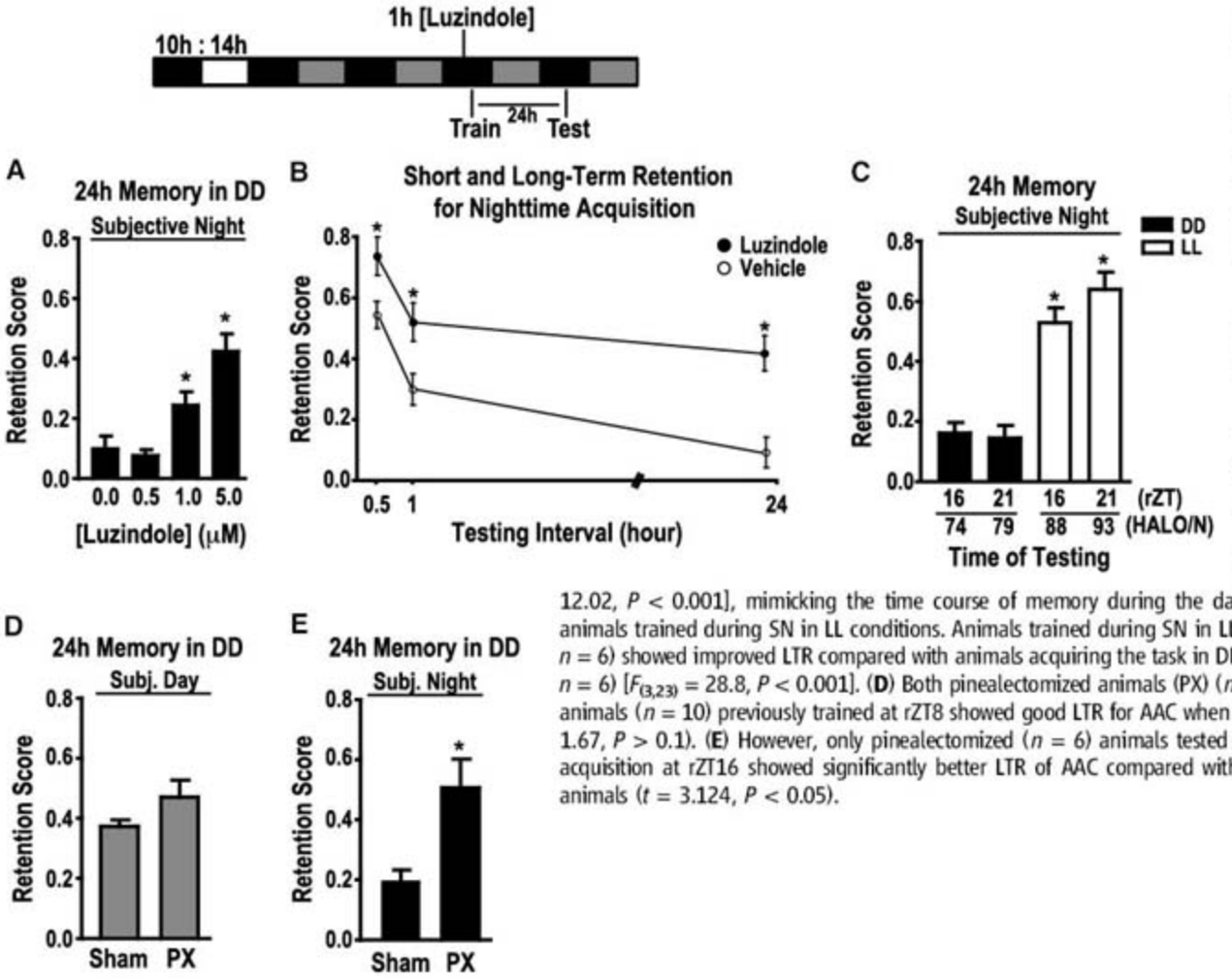

Fig. 3. Melatonin is necessary for poor nighttime LTM. (**A**) Dose-dependent effect of luzindole during SN on LTM formation. Animals previously treated with luzindole at rZT16 (*5*) and tested 24 hours later showed improved LTR for AAC [$F_{(3,15)} = 13.13$, $P < 0.001$; Newmann-Keuls posthoc analysis for control versus 0.5 μM, $P > 0.05$, and for control versus 1.0 μM and 5.0 μM, $P < 0.05$]. (**B**) Blocking melatonin signaling at night improved 30-min, 1-hour, and 24-hour memories [$F_{(5,30)} = 12.02$, $P < 0.001$], mimicking the time course of memory during the day. (**C**) LTM formation for animals trained during SN in LL conditions. Animals trained during SN in LL (rZT16, $n = 6$, or rZT21, $n = 6$) showed improved LTR compared with animals acquiring the task in DD (rZT16, $n = 6$, or rZT21, $n = 6$) [$F_{(3,23)} = 28.8$, $P < 0.001$]. (**D**) Both pinealectomized animals (PX) ($n = 9$) and sham-operated animals ($n = 10$) previously trained at rZT8 showed good LTR for AAC when tested 24 hours later ($t = 1.67$, $P > 0.1$). (**E**) However, only pinealectomized ($n = 6$) animals tested 24 hours after nighttime acquisition at rZT16 showed significantly better LTR of AAC compared with sham-operated ($n = 6$) animals ($t = 3.124$, $P < 0.05$).

mation when presented during the SD. Inhibiting the rise in melatonin during the SN through constant light, pinealectomy, or blocking physiological melatonin signaling all lead to significantly improved LTM formation.

The effect of melatonin on nighttime memory formation may be through the direct modulation of memory formation circuits. Melatonin receptors are widely distributed in the vertebrate brain (*16*), and melatonin has been shown to modulate neuronal firing in the hippocampus (*8*, *9*, *17*). Furthermore, both diurnal as well as circadian differences in hippocampal long-term potentiation have been reported (*18*). Thus, melatonin could affect nighttime memory formation by directly altering neural excitability in zebrafish. Alternatively, melatonin may act indirectly by affecting sleep or circadian phase requirements for memory formation. Nighttime sleep is considered to be an important mediator for memory consolidation. However, we do not believe that disturbances to sleep architecture played a significant role here because animals trained early during the night at rZT16 had 7 to 8 hours of the original subjective nighttime left before onset of activity and showed LTR similar to animals trained at mid- or late night (Fig. 1D). Furthermore, it is unlikely that the poor LTM for animals trained during the SN was due to the effect of sleep disturbance on memory retrieval when tested 24 hours later because animals tested 48 hours and 72 hours after training, allowing enough recovery time, did not show a significant improvement in LTM (Student's $t = 0.325$, $P > 0.5$).

Zebrafish is a diurnal species with several fundamental similarities to humans. Both show a similar melatonin secretion profile with respect to the organism's sleep-wake cycle; also melatonin's role in sleep and possibly other physiological and behavioral effects appear conserved (*19*). Therefore, our finding that nighttime melatonin may actively suppress memory consolidation after nighttime acquisition suggests a similar endogenous role for melatonin in humans, especially because several studies have shown that changes in cognition and behavior are concurrent to abnormalities in melatonin rhythms in humans (*20*). The finding that memory can be improved by blocking melatonin signaling during the night encourages further research into melatonin signaling for therapeutic treatment to improve mental performance.

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Supporting Online Material

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Fast-Forward Playback of Recent Memory Sequences in Prefrontal Cortex During Sleep

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As previously shown in the hippocampus and other brain areas, patterns of firing-rate correlations between neurons in the rat medial prefrontal cortex during a repetitive sequence task were preserved during subsequent sleep, suggesting that waking patterns are reactivated. We found that, during sleep, reactivation of spatiotemporal patterns was coherent across the network and compressed in time by a factor of 6 to 7. Thus, when behavioral constraints are removed, the brain's intrinsic processing speed may be much faster than it is in real time. Given recent evidence implicating the medial prefrontal cortex in retrieval of long-term memories, the observed replay may play a role in the process of memory consolidation.

According to memory-consolidation theory, the hippocampus is necessary for the retrieval of recently encoded episodic memories. For remote memories, in contrast, the neocortex is sufficient for recall (*1*–*4*). The transfer of memories from hippocampal to neocortical control is widely believed to involve replay during sleep of the neural patterns representing the memory (*5*–*7*). Consistent with this hypothesis, patterns of brain activity during a task appear to be repeated during subsequent sleep in rats, birds, monkeys, and humans (*8*–*16*). In rats, multielectrode hippocampal recordings have shown that the temporal order of replay during sleep is preserved (*17*, *18*).

Among cortical areas, the medial prefrontal cortex (mPFC) apparently plays a unique role in mediating retrieval of consolidated, remote memories. In both rats and humans, activity in the mPFC is greater during retrieval of remote memories than during retrieval of recent memories; an opposite pattern is seen in the hippocampus (*19*–*21*). In addition, lesions of mPFC lead to deficits in retrieval of remote memories (*19*, *20*, *22*, *23*). One might thus expect the mPFC to play a key role in memory consolidation. Indeed, disrupting mPFC activity during consolidation impairs subsequent performance (*24*).

If mPFC does play a role in consolidation, task-induced neural activity patterns in mPFC may replay during subsequent sleep. We therefore examined neural ensemble activity after performance of a spatial sequence task. Two rats were trained to run to a series of locations around the perimeter of a 1.3-m circular platform with electrical brain stimulation as a reward. Sequences, consisting of eight locations, were repeated throughout the course of a 50-min running session, alternating in blocks of three cued and three noncued (i.e., memory-guided) sequences throughout the session. Each day, a rat ran the sequence task continuously during two 50-min blocks. Neural activity was recorded during a pre-task sleep period preceding the first task block and in two post-task sleep periods following each task block. Each sleep session was 20 to 60 min in duration. Both rats were implanted with microdrives containing 12 independently manipulable four-conductor electrodes ("tetrodes") (*25*), allowing simultaneous recording of 40 to 120 neurons within the anterior cingulate and prelimbic cortices. Memory reactivation was assessed during periods of motionlessness during the sleep session.

The reactivation of the task-related neural patterns was initially assessed with a measure called "explained variance" (EV), based on the firing rate correlation matrix for all pairs of concurrently recorded cells (*26*). Explained variance measures the proportion of variability in the cell-pair firing-rate correlations during task performance that can be accounted for by correlations during subsequent sleep, taking into account the correlations that existed in the initial sleep session. For this measure, 100% would mean that cell-pair correlations during task and subsequent sleep were identical. Previous reactivation studies have found average EV values of ~15%

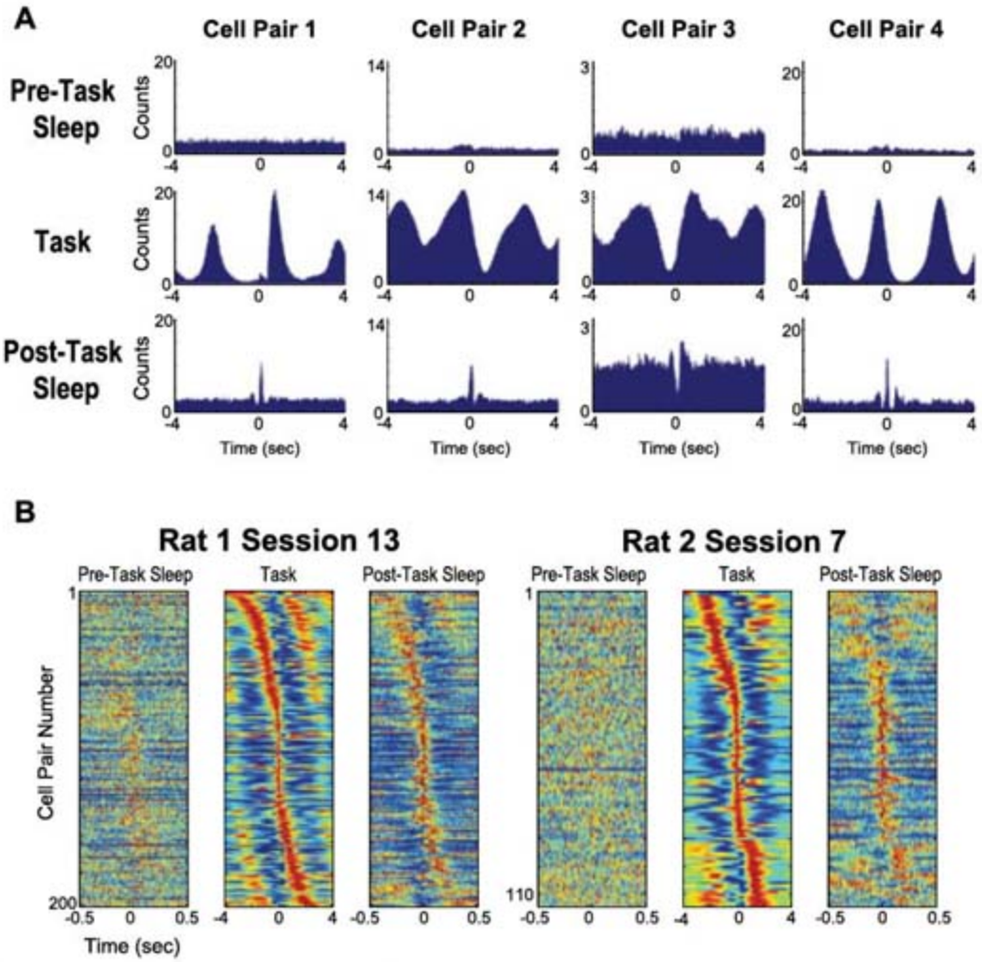

Fig. 1. Cross-correlations between mPFC cell pairs during task and sleep. (**A**) Example cross-correlations from four cell pairs. Each column shows data from one cell pair during pre-task sleep, task, and post-task sleep. The *y* axis shows the number of coincident spikes per second within each 10-ms bin. (**B**) Sorted cross-correlations from simultaneously recorded cell pairs. Each row in each subpanel shows the cross-correlation between a single pair of cells, scaled so that peak and valley range from zero to one. The rows are sorted according to the temporal offset of the maximum peak during the task. In addition, only cell pairs showing a peak *z* score exceeding 11 during the task were included (~5 to 15%; see Supporting Online Material for details). Red indicates the highest coincidence rate and blue, the lowest. The time axis during sleep epochs is magnified.

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in rat hippocampus, ~10% in rat ventral striatum, and between 5 and 11% in monkey cortex (*13*, *14*, *26*). Average EV in the current study was 11% across two rats and a total of 66 sessions. The distribution of values was strongly skewed; the average of EV values within the lower quartile was 4% and that in the upper quartile, 22%. Reactivation was significantly stronger in the sleep following the second task period than in the sleep following the first (6% versus 11%, Student's t test, $P < 0.01$). At least in some sessions, reactivation in mPFC was as strong as, if not stronger than, that reported in the hippocampus or other parts of the brain.

Cross-correlations of spike trains from pairs of cells were used to examine the temporal structure of replaying patterns in mPFC. Analyses were limited to the second post-task sleep, which is the period showing strongest EV. Cells in mPFC tend to fire at specific locations along a segment, with the majority firing selectively during either approach to or departure from at least some reward zones. These response characteristics, combined with the repetitive nature of the task, led to distinct peaks and valleys in many of the cross-correlation plots (Fig. 1A), indicating consistent temporal relationships in neuronal firing. For some cell pairs, the increased number of central peaks and valleys seen during the task were evident again in the post-task sleep, but highly compressed in time, suggesting that temporal patterns playing during the task were replaying during sleep at an accelerated rate. Peaks and valleys were small or nonexistent during pre-task sleep, suggesting that the replaying patterns were induced by task-related activity. Similar results were obtained when data from multiple cell pairs within the same session were compared together (Fig. 1B).

To quantify the extent to which neural patterns during sleep evolved at a faster rate than those during the task, times of cross-correlation peaks during task and sleep were automatically extracted and compared (Fig. 2A). For peaks nearest the origin, a strong linear relation was found between task and sleep peak times, with slopes indicating that replay during sleep is compressed relative to the task. Because this analysis requires many cell pairs with matching task and sleep cross-correlations, compression rates were only computed for sessions where the explained variance exceeded 15%, a total of 13 sessions (rat 1: 10; rat 2: 3). Compression rates varied between 5.4 and 8.1, with a mean of 6.5 (Fig. 2B).

The temporal extent of replay is limited. The attenuation of a given peak during sleep seems to be proportional to the offset of the corresponding peak during the task, with peaks beyond about 3 s being almost completely lost (Fig. 1A). To quantify the duration of the replay window, we compared sleep and compressed task cross-correlations at different offset times (Fig. 3A). The correlation between sleep and task cross-correlations was strongest near zero and then dropped off rapidly, reaching near chance levels around 750 ms (Fig. 3B). This suggested that replaying patterns were, on average, coherent for a duration of ~1.5 s (2 × 750 ms) during sleep. Assuming 6.5 times compression, the mPFC was evidently replaying behavioral events spanning ~10 s during the task (about half of a complete eight-element sequence).

Template matching (*18*) was used to assess whether the replay evident in pairwise activity was coherent across ensembles of cells. For each of the eight sequence segments, we created a template by averaging binned firing rates of a group of cells across multiple repetitions of the sequence (Fig. 4A, left). As expected, many strong matches to each template were observed during the task. Strong matches were also observed during post-task sleep, but only when sleep bin size was reduced, effectively compressing the template relative to sleep (Fig. 4A, right). Indeed, the highest number of strong matches occurred for compression factors of 6 to 7 (Fig. 4C), in accord with estimates based on matching cross-correlation peaks. Few matches were observed when the template column order was reversed, indicating that replay occurs in the forward direction.

Whether the observed mPFC replay during the first hour of sleep represents off-line rehearsal needed for memory recoding remains an open question; however, the time frame coincides with what other research suggests is a critical window for memory processing. Studies in humans have shown that memory is tied to slow-wave activity within the first few hours of sleep (*16*, *27*). In rats, local injection of drugs disruptive to mPFC function leads to learning deficits when given within 2 hours after a task, but not after this time (*24*, *28*).

The hippocampus reportedly replays events at 5 to 20 times their behavioral rate (*17*, *29*–*31*);

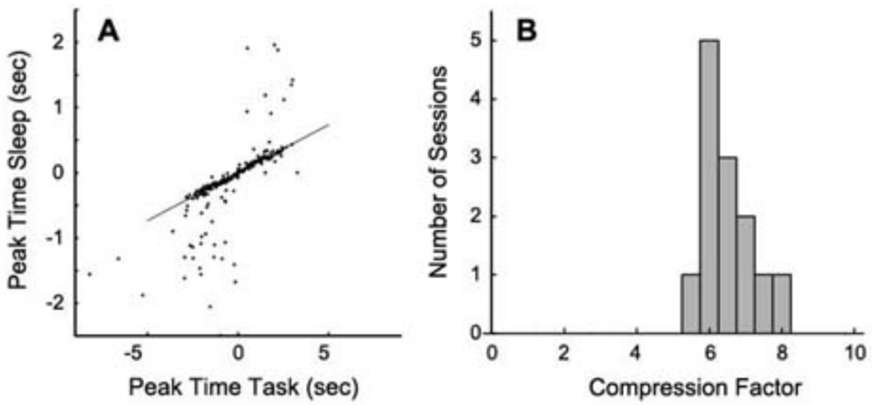
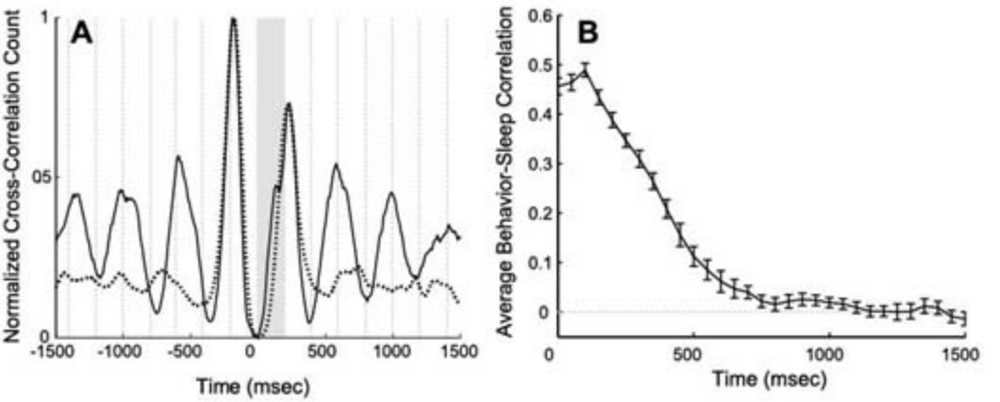


Fig. 2. Quantification of temporal compression during reactivation. (**A**) Task cross-correlation peak times plotted against post-task sleep peak times. In each case, two peaks are extracted from each cross-correlation, one on the left of zero and one on the right. Matches in the upper left and lower right quadrants of the graph are thus precluded. Estimating compression rate depended upon finding corresponding peaks. Therefore, cell pairs were limited to those showing strong similarity between task and sleep cross-correlations after accounting for compression (i.e., cell pairs exhibiting strong reactivation). The best-fit regression line was found with robust regression, a technique less sensitive to outliers than normal regression. (**B**) Histogram of compression rates extracted with the analysis shown in (A).

Fig. 3. Temporal extent of replay window. (**A**) Cross-correlations from post-task sleep (dashed line) and task (solid line) were compared with the use of a sliding 200-ms window (gray bar). In this example, the task cross-correlation has been compressed by a factor of 6.8. (**B**) Strength of the correlation between task and sleep cross-correlations in each window plotted against time (as measured with the sleep time frame). Data shown were averaged across all 13 high reactivation sessions, with vertical bars indicating the SEM. Data from negative time windows were averaged with the corresponding positive time windows.



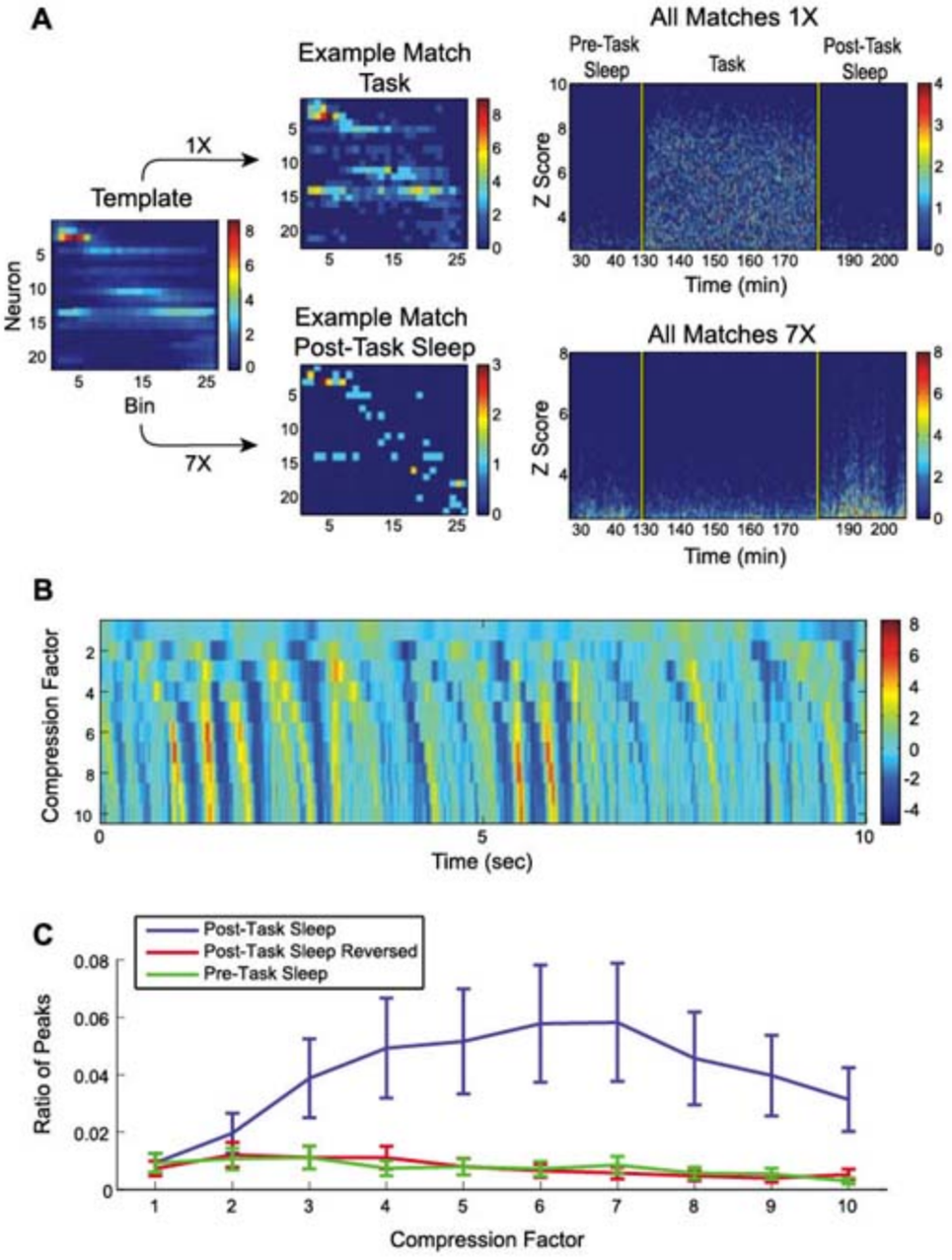


Fig. 4. Template matching. (**A**) (Left) Template from one segment of the sequence, showing firing rate (spikes/bin) from multiple cells (*y* axis) sorted by time of peak firing (*x* axis). (Middle) Examples of good matches to the template from sleep (bottom) and task (top) periods. Bin size for the task and sleep examples are 100 ms and 14 ms respectively, the latter representing a compression factor of 7. (Right) The two graphs show histograms of match strength (*z* scores) between templates and "target" data within sequentially ordered 14-s windows. *Z* scores were derived via random shuffling of template columns. Color indicates the number of matches. In the top graph, template and target bin size are the same. In the bottom graph, target bin size is a factor of 7 smaller than in the template. The time axis is discontinuous because first task and second sleep period were omitted. (**B**) Match strength between template and a portion of post-task sleep data at different compression factors (indicating the extent to which sleep replay is compressed relative to the task). Most of the strong matches (i.e., red vertical bands) had peaks corresponding to compression factors between 6 and 8. (**C**) For each compression factor, the number of local maxima (i.e., peaks) with a *z* score exceeding 4 was divided by the total number of peaks ("Ratio of Peaks"). Data are averaged over all eight templates with the SEM indicated. Neither post-task sleep with reversed templates nor pre-task sleep showed strong matches or evidence of a peak compression rate.

however, in the hippocampus, spikes representing adjacent place fields occur in rapid succession within a single theta cycle during behavior (*32*). Relative to this within–theta cycle rate, reactivation during sleep is not accelerated. In contrast, reactivation in rat mPFC is clearly compressed five to eight times, and a similar effect may be present in primary visual cortex (*31*). Fast replay in neocortex may reflect the speed of the brain's intrinsic dynamics (e.g., conduction speeds, synaptic delays, etc.) when not constrained by behavioral events. However, the episodes of sequential replay during sleep are limited to windows of a few hundred milliseconds. This may reflect a time limitation imposed by the duration of cortical "up" states induced by slow-wave oscillations (*33*) during which replay presumably occurs, or it may reflect cumulative drift error in the sequence replay.

In conclusion, two independent analytical methods, cross-correlation matching and template matching, both clearly showed accelerated replay of task-related neural activity patterns within the mPFC during sleep. That this happens (i) in an area implicated in remote memory retrieval and (ii) during a time window in which processes critical to consolidation are unfolding suggests that accelerated replay may be an important part of the process whereby hippocampus-dependent memories become cortex-dependent.

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Supporting Online Material

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Fig. S1
Table S1
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Time-Dependent Central Compensatory Mechanisms of Finger Dexterity After Spinal Cord Injury

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Transection of the direct cortico-motoneuronal pathway at the mid-cervical segment of the spinal cord in the macaque monkey results in a transient impairment of finger movements. Finger dexterity recovers within a few months. Combined brain imaging and reversible pharmacological inactivation of motor cortical regions suggest that the recovery involves the bilateral primary motor cortex during the early recovery stage and more extensive regions of the contralesional primary motor cortex and bilateral premotor cortex during the late recovery stage. These changes in the activation pattern of frontal motor-related areas represent an adaptive strategy for functional compensation after spinal cord injury.

Neurorehabilitation has its basis in the concept that training recruits the remaining neuronal systems to compensate for partial injury of the central nervous system (CNS). However, the neuronal basis of these compensation mechanisms is poorly understood. Brain imaging studies in human stroke patients show increased activity in various cortical regions, including the side ipsilateral to the affected extremity (*1*, *2*). However, in these case studies, the extent of the lesion varies between patients, and thus identifying the damaged pathways is often difficult. Moreover, it is unclear whether the brain regions showing increased activity causally contribute to the recovery. In addition, because the tests are usually performed at a specific time after the lesion, longitudinal information is lacking. To assess the neuronal mechanism of functional compensation, we need a longitudinal study that applies quantitative behavioral evaluation to an animal model, preferably macaque monkeys (*3*), with a defined lesion of the particular neuronal system. Dexterous finger movements can be restored within a few weeks to 1 to 3 months after a lesion of the direct cortico-motoneuronal (CM) connection via the lateral corticospinal tract (l-CST) at the border between the C4 and C5 segments of the spinal cord (*4*). This lesion site ("C4/C5 l-CST lesion") is rostral to the segments where motoneurons of hand muscles are located. These results suggest that indirect cortico-motoneuronal pathways, mediated by subcortical or spinal interneuronal systems, can mediate commands for the control of dexterous finger movements in primates. In the present study, we examined the neuronal mechanism of this functional recovery from spinal cord injury. We hypothesized that, in addition to the plasticity of neural circuits in the spinal cord (*4*, *5*), adaptive learning by higher order structures may contribute to the recovery. We applied positron emission tomography (PET) scanning using $H_2{}^{15}O$ to measure changes in brain activity during precision grip tasks at different stages of recovery.

Five monkeys were trained to reach for a small piece of food through a narrow vertical slit and to grasp it between the pads of the index finger and thumb (Fig. 1A, preop). In the monkey shown in Fig. 1A, the precision grip was completely impaired immediately after the C4/C5 l-CST lesion (Fig. 1A, day 7). On day 14, the monkey was able to grasp the food with the index finger and thumb, but the independence of the fingers remained impaired. All abilities gradually recovered (Fig. 1A, day 99), as previously reported (*4*). Figure 1B shows the time course of recovery of the success rate for precision grip. The success rate recovered to more than 80% of that before the lesion within 3 weeks in all five monkeys. On the basis of these observations, we defined postoperative days 1 to 45 (about 1 month postoperative) as the early recovery stage and postoperative days 90 to 143 (more than 3 months) as the late recovery stage. We performed PET scanning and inactivation experiments during the following three stages: (i) preoperative stage, (ii) early recovery stage, and (iii) late recovery stage.

Three monkeys (monkeys H, T, and K) were examined in the PET study. In multiple comparisons, their performance in the precision grip task during the preoperative stage was associated with an increased activity in visuomotor-related regions, including the sensorimotor cortex, premotor cortex, and intraparietal sulcus in the contralateral hemisphere and the early visual cortices, putamen, and the cerebellum on the ipsilateral side, as previously shown (*6*) (Fig. 2). To identify the cortical regions that showed an increase in activity during postoperative stages, we compared the regional cerebral blood flow (r-CBF) during the postoperative stages with that during the preoperative stage. Activity increased in the bilateral primary motor cortex (M1) during early recovery (Fig. 3, A to C and E, and table S1). Furthermore, activity increased in the bilateral early visual cortices (V1/V2), contralateral S2, contralateral accumbens, and the vermis of the cerebellar cortex (Fig. 3, A to G, and table S1). During the late recovery stage, increased activation was observed in the contralateral M1 (co-M1) and ipsilateral ventral premotor cortex (ip-PMv) (Fig. 3, I to M, and table S1). Activity in the bilateral insula, contralesional accumbens, and the cerebellar vermis also increased (Fig. 3, L to O, and table S1). The area of co-M1 with increased activity expanded during the late recovery stage compared with that during

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the early recovery stage (compare Fig. 3, A and I, B and J, and E and M), and the increased activity extended into co-PMv (Fig. 3J and table S1).

To clarify whether the increased activity of those cortical regions observed in the PET study were causally involved in the functional recovery, we performed focal inactivation of individual cortical regions by using microinjections of muscimol, a γ-aminobutyric acid type A ($GABA_A$) receptor agonist (5 μg/μl), at various recovery stages and observed the precision grip in two monkeys (monkeys S and C). Because we observed increases in the activity in M1 and PMv of both hemispheres during the recovery stages in the PET study, we chose the digit areas of these cortical regions as targets of muscimol injection. We performed intracortical microstimulation (ICMS) mapping before the inactivation study to obtain a topographical map of these regions and to determine the injection site (Fig. 4A, a and b). In the preoperative trials, inactivation of the digit area of co-M1 with 0.8 or 1.5 μl of muscimol resulted in clumsy finger movements, accompanied by a loss of the independent control of each digit. Both monkeys reached for the food piece but were not able to achieve a precision grip. The success rate for retrieval was reduced to zero (Fig. 4B, d and j). On the other hand, inactivation of either ip-M1 (Fig. 4B, a and g, 5.0 μl of muscimol) or co-PMv (Fig. 4B, p and v, 1.5 μl of muscimol) or ip-PMv (Fig. 4B, m and s, 5.0 μl of muscimol) resulted in no impairment of digit movements. Precision grip during the early recovery stage was severely impaired by inactivation of co-M1. The monkeys reached for the target but showed muscular hypotonia in the hand. Monkey C showed total paresis of the hand. Monkey S was able to move its digits, but the thumb and index finger could not be inserted into the slit. The success rate for retrieval remained zero in both monkeys (Fig. 4B, e and k). Interestingly, after inactivation of ip-M1, the capacity to retrieve the food piece was impaired. The success rate for retrieval with precision grip in monkeys S and C decreased by 33 and 15%, respectively (Fig. 4B, b and h), from that before inactivation. Even in successful trials, both monkeys achieved the grip not with the pads of index finger and thumb but with the pad of index finger and the nail of thumb. After inactivation of co-PMv (Fig. 4Bq) or ip-PMv (Fig. 4Bn), the capacity to retrieve the food piece was impaired, and digit movements became clumsy in monkey C but not in monkey S. During the late recovery stage, the effect of inactivation of co-M1 was greatly reduced, even in comparison with the preoperative trials (Fig. 4B, f and l). In contrast to the early stage, inactivation of ip-M1 during the late recovery stage resulted in no impairment of digit movement in either monkey (Fig. 4B, c and i). Inactivation of co-PMv did not cause impairment of digit movements in either monkey (Fig.

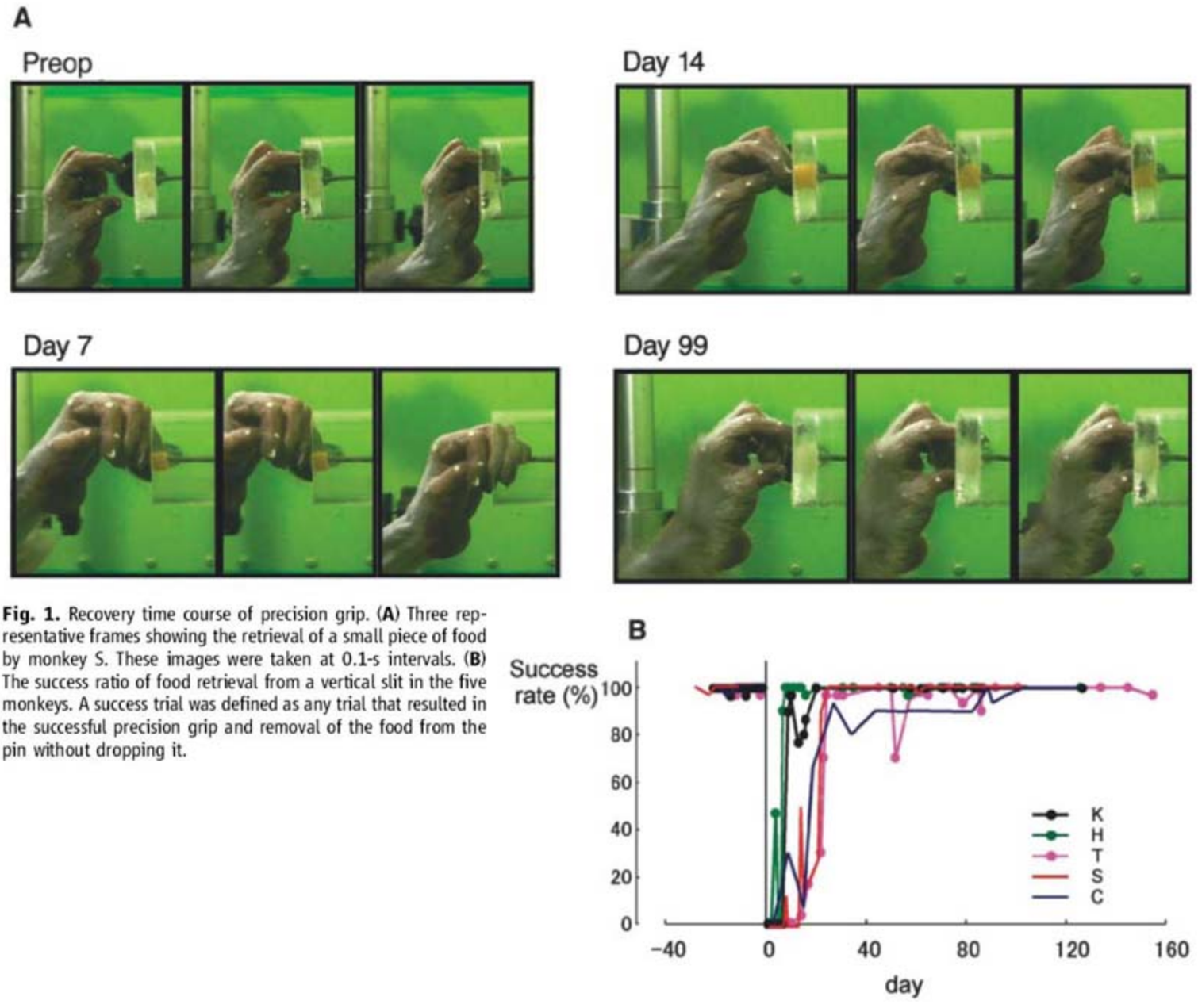

Fig. 1. Recovery time course of precision grip. (**A**) Three representative frames showing the retrieval of a small piece of food by monkey S. These images were taken at 0.1-s intervals. (**B**) The success ratio of food retrieval from a vertical slit in the five monkeys. A success trial was defined as any trial that resulted in the successful precision grip and removal of the food from the pin without dropping it.

3B, r and x). We confirmed these results by administering additional injections of muscimol into ip-M1 or co-PMv, and no impairment of digit movements was observed. Inactivation of ip-PMv led to a marked slowing of movements in both monkeys (Fig. 4Bu). Monkey C repeated several grips before retrieving the food piece.

During surgery, we tried to achieve a complete lesion of the direct CM connection, leaving the ventrally located interneuronally mediated pathways intact (fig. S1A). The extent of the lesion was similar among all five monkeys (fig. S2). The extent of the lesion was assessed by counting the number of anterogradely labeled l-CST axons caudal to the lesion in monkey T (fig. S1C) versus rostral to the lesion (fig. S1B) after biotinylated dextran amine (BDA) injection into the co-M1. A small number of corticospinal fibers remained in the ventral region (box in fig. S1C). Thus, the extent of interruption of the l-CST fibers was estimated to be 98.7%. The extent of the lesion was also assessed by electrophysiological recordings in three monkeys (monkeys T, H, and S) under anesthesia. Extracellular field potentials in the lateral motor nuclei in C6 (a train of three stimuli was applied to the contralateral medullary pyramid) and the cord dorsum potential (CDP) at the same segment on the intact and lesion sides were recorded (fig. S3). The extent of the lesion estimated by the amplitude of the negative volley in CDP was 96.4% in monkey T, 100% in monkey H, and 99.6% in monkey S (relative to the intact side). Thus, we conclude that a near-complete or complete lesion was made to l-CST in all five monkeys.

Our results indicate that the inactivation of co-M1 resulted in a severe deficit in finger movements during the early recovery stage (Fig. 4B, e and k). This suggests that early recovery depends strongly on increased activity of the co-M1. Interestingly, during the late recovery stage, the effect of inactivation of co-M1 was greatly reduced in comparison with the early recovery period (Fig. 4B, f and l). As shown in Fig. 3, I, J, and M, the activated area in the co-M1 greatly expanded and appeared to extend into co-PMv. Additionally, increased activation was found in ip-PMv. The present result suggests that the recovery process was also assumed by regions outside the preoperative digit area in co-M1 and that the effect of inactivation of the digit area by injection of the same amount of muscimol could be compensated by the strong activity in these surrounding regions and ip-PMv. Our finding of an increased area of activation in co-M1 agrees with previous observations that the representation of trained movement in M1 expanded with learning (*7*, *8*) and with recovery after injury (*9*–*11*). Neuronal pathways from these activated areas to the hand motoneurons are not clear. Propriospinal neurons with cell bodies in the C3-C4 segments and with axons passing through the ventral part of the lateral funiculus can mediate the excitation from the co-M1 to digit motoneurons (*12*–*14*). However, the contribution of reticulospinal neurons cannot be excluded (*15*, *16*).

The inactivation of ip-M1 resulted in no deficit in the preoperative trials but caused a deficit during the early recovery stage (Fig. 4Bb) and no deficit during the late recovery stage (Fig. 4Bc). Ip-M1 thus transiently contributes to the recovery during the early recovery stage. Increased activity in M1, premotor area, and supplementary motor area on the ipsilateral side to the affected hand has been reported in stroke patients (*1*, *2*). The activation of ip-M1 was reported to have a negative correlation with the outcome after stroke (*1*). However, in the above studies, it was not clear whether the increased activity was controlling the digit movements or was simply a side effect of the increased activity of the co-M1. Inactivation of ip-M1 after hemisection at the cervical spinal cord of the monkey produced no effects (*17*). However, these examinations were performed over 3 months after the lesion, which may have been too late to show effects, judging from our observation presented herein. The ip-M1 may exert effects either through the co-M1 via callosal fibers (*18*–*20*) or by relay through subcortical pathways (*15*, *16*, *21*, *22*). Furthermore, axons descend through the l-CST that originate from the ip-M1 and recross the midline at the spinal level (*23*). This commissural projection may also drive hand motoneurons. Similar indirect excitatory pathways from the ip-M1 to hand motoneurons may exist. Transmission through these pathways should be

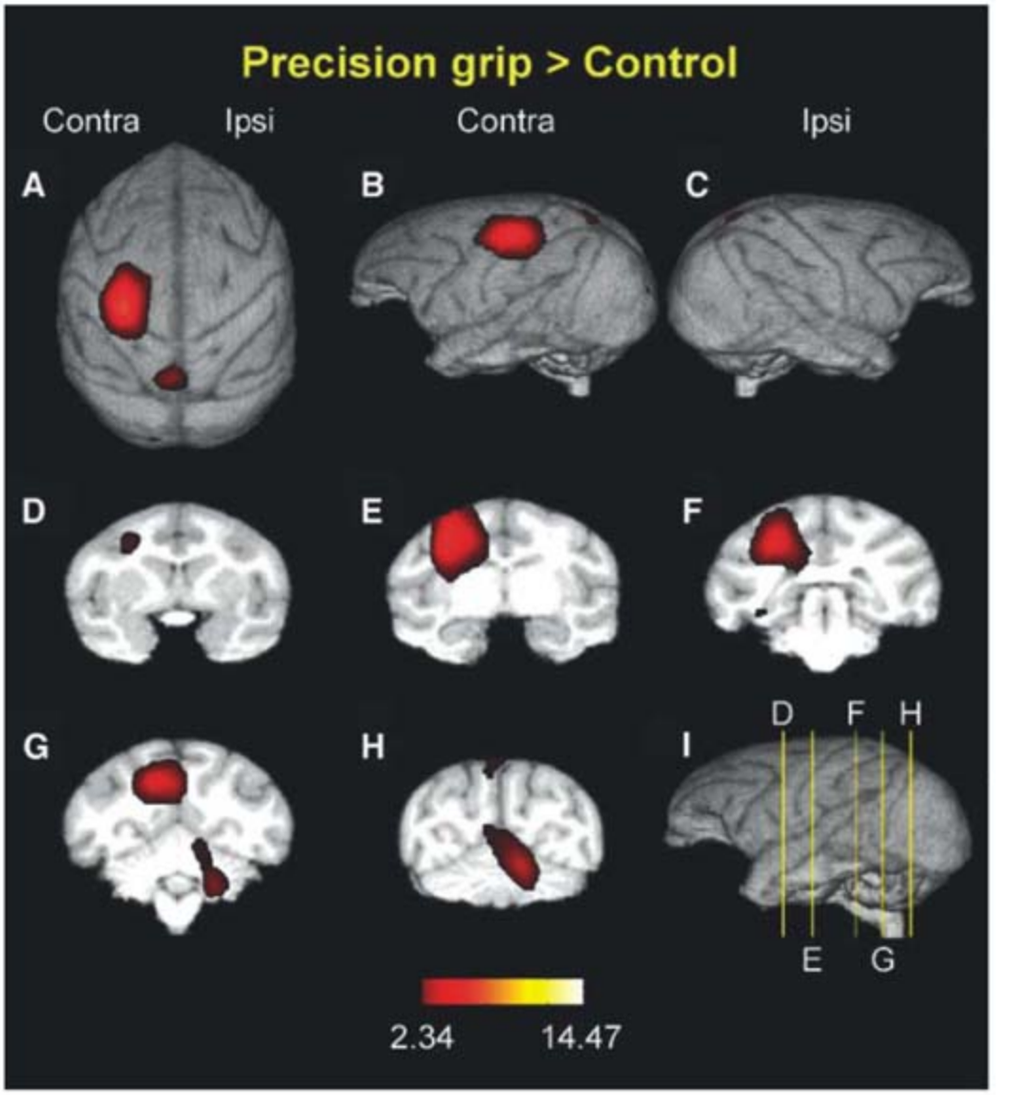

Fig. 2. Brain areas activated during the precision grip task at the preoperative stage were quantified as increased rCBF using PET. rCBF on the control task at the preoperative stage was subtracted from rCBF on the precision grip task at the preoperative stage. Brain areas with significantly increased rCBF are indicated ($P < 0.01$, uncorrected for multiple comparisons in three monkeys). The activations are superimposed on a three-dimensional reconstruction of a template brain magnetic resonance image (MRI) of macaque monkeys that was produced by our group. The significance level is given in terms of a *Z* score represented on a colored scale. (**A**) Top view, (**B**) view from the contralesional hemisphere, (**C**) view from the ipsilesional hemisphere, (**D** to **H**) coronal sections, and (**I**) lateral view of the brain. The lines D to H in (I) correspond to the respective coronal sections. Contra indicates contralesional hemisphere; Ipsi, ipsilesional hemisphere.

inhibited normally but may be disinhibited after injury (*24*).

A contribution from the PMv has been indicated for the recovery from an M1 lesion through observations of changes in the topographic map (*25*). Concerning the co-PMv, it has been shown to influence motoneuronal activity mainly via the co-M1 (*26*, *27*). The co-PMv may also control the spinal interneuronal system directly via its direct projection to the mid-cervical segments (*28*). The pathway from the ip-PMv is difficult to explain; however, it is likely that the ip-PMv can exert its effect via the co-PMv, co-M1 (*18*–*20*), or a direct projection to the spinal cord and commissural projection at the spinal level.

The present study demonstrates that functional recovery after lesion of the corticospinal tract involves specific networks, including not only interneuronally mediated subcortical pathways downstream of the co-M1, but also other parallel pathways including the ip-M1 and higher-order cortices like the PMv. The contribution of each cortical region changes depending on the postoperative recovery stage. We thus suggest that the brain uses existing systems by reducing inhibition during the early recovery stage and gradually enhancing the original neural systems or recruiting other systems by synaptic plasticity during the late recovery stage for more

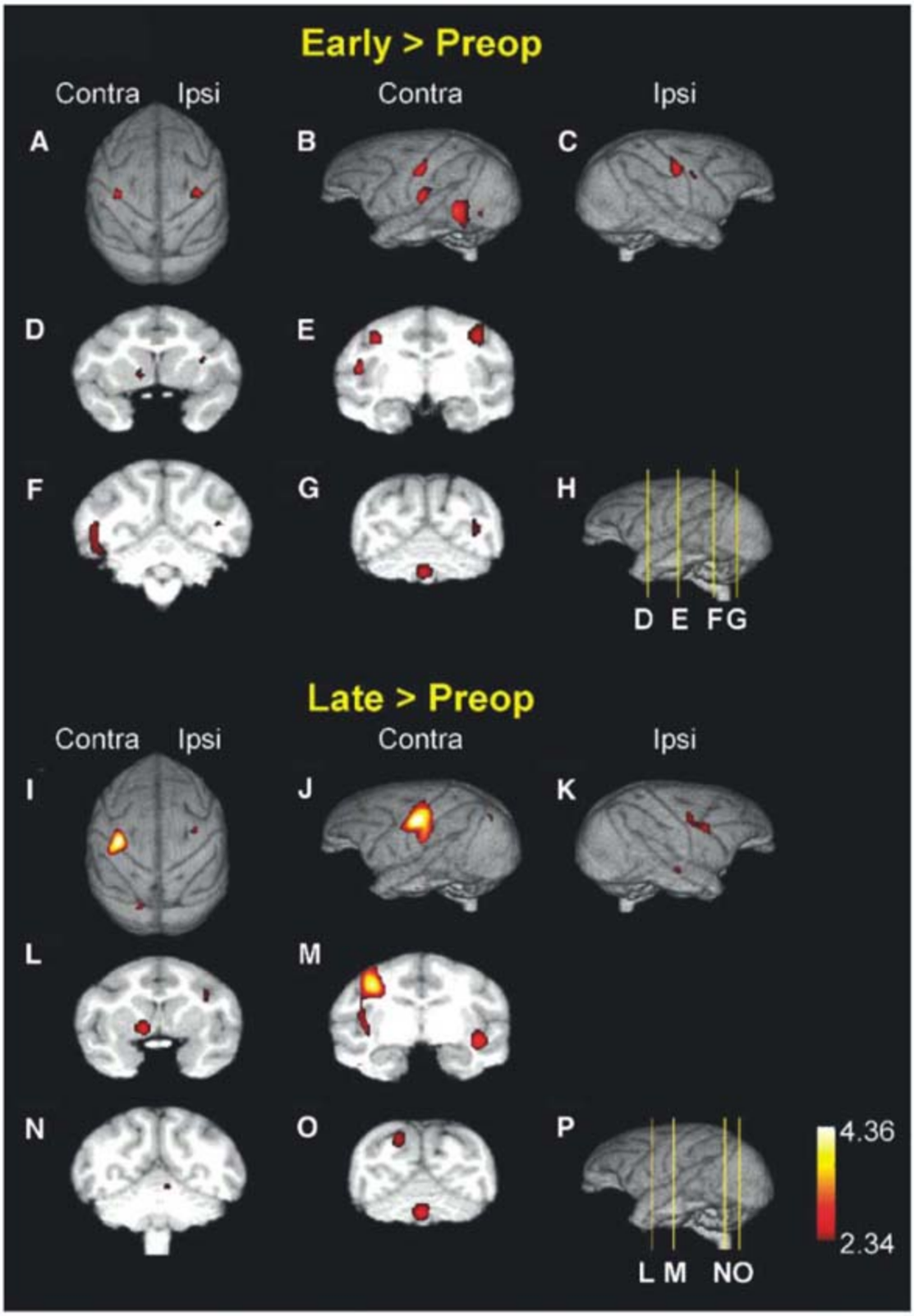

Fig. 3. Increased brain activation related to functional recovery. Results were obtained from three monkeys (monkey H, T, and K). Twenty scans were conducted on monkeys H and K in both tasks for every stage, and 24 scans were conducted on monkey T. Activation during early and late recovery stages was compared with the preoperative stage (precision grip task at postoperative stage − control task at postoperative stage) − (precision grip task at preoperative stage − control task at preoperative stage). Brain areas with significantly increased rCBF ($P < 0.01$, uncorrected for multiple comparisons) are superimposed on a three-dimensional reconstruction of a template brain MRI of macaque monkeys that was made by our group. The significance level is given in terms of a Z score represented on a colored scale. (**A** to **G**) and (**I** to **O**) are results during early and late stages of recovery, respectively. (A) and (I), top view; (B) and (J), view from contralesional hemisphere; (C) and (K), ipsilesional hemisphere; (D) to (G) and (L) to (O), coronal sections. (**H**) and (**P**) show lateral views of the brain. Lines D to G and L to O in (H) and (P) indicate the levels of coronal sections of (D) to (G) and (L) to (O), respectively.

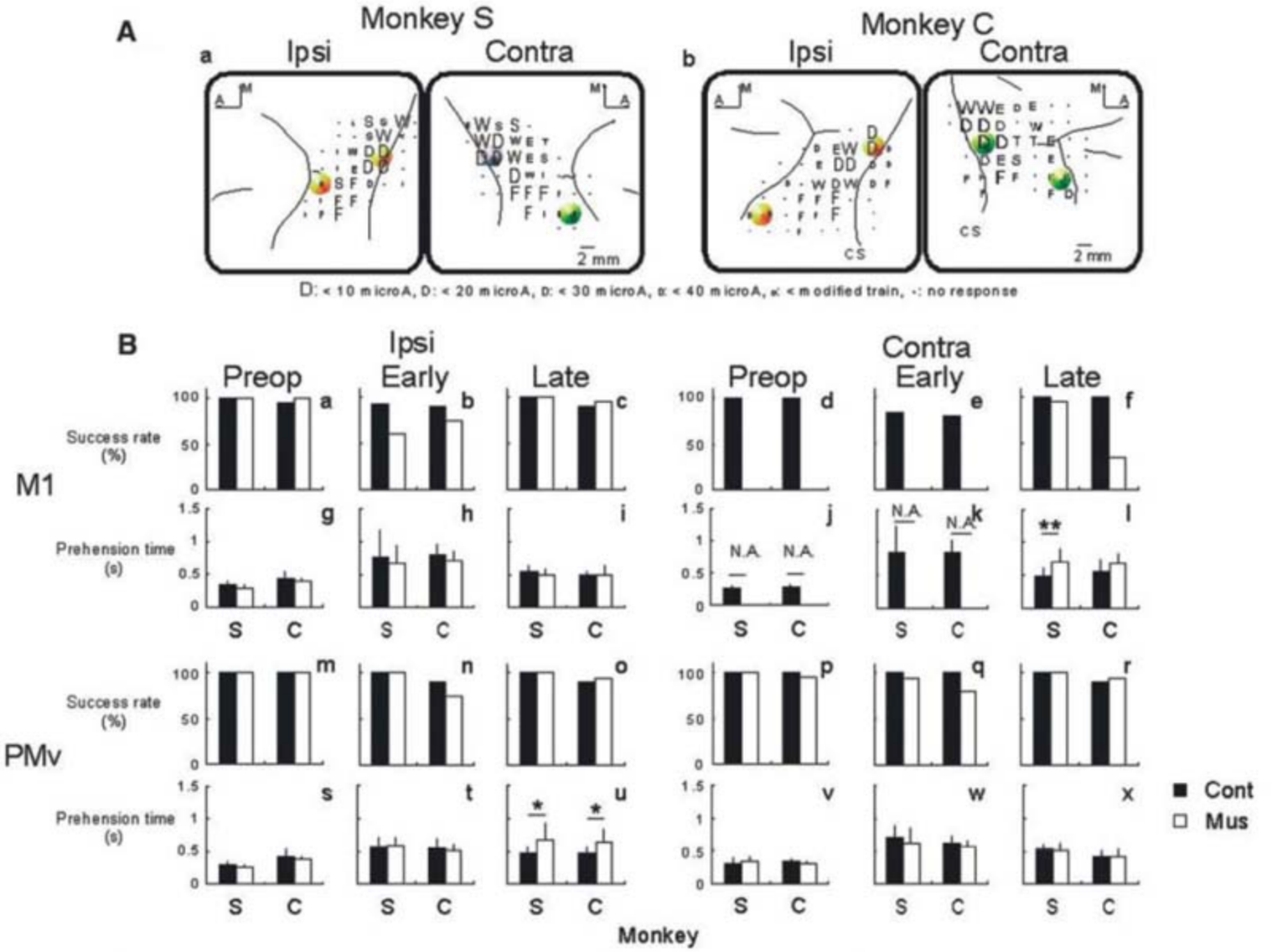

Fig. 4. The effect of inactivation of M1 or PMv on the precision grip. (**A**) Somatotopic map revealed by ICMS performed before the CST lesion. The location of muscimol injections are indicated on a surface map of the precentral regions shown in a and b. Each electrode penetration is represented with a character indicating the body territory activated at threshold: D, digit; W, wrist; E, elbow; S, shoulder; T, trunk; and F, face. The size of characters indicates the threshold for induction of movements (inset). The sites and volumes of muscimol injection are shown by colored circles (concentration, 5 μg/μl; volume, light purple dot, 0.8 μl; green dots, 1.5 μl; orange dots, 5.0 μl). Anterior-posterior and medial-lateral orientation are indicated in the inset (A, anterior; M, medial). (**B**) The effect of inactivation of M1 or PMv on food retrieval at preoperative, early, and late recovery stages in two monkeys. The success rates for target retrievals and retrieval time obtained before (Cont) and after muscimol injection (Mus, 2 hours after muscimol injection). The prehension time was defined as the time interval between the first insert of any digit to the tube and the timing of release of all the digits from the tube. Only trials with successful retrieval were used to measure the prehension time. a to c and g to i, ip-M1; d to f and j to l, co-M1; m to o and sto u, ipsilesional PMv; p to r and v to x, co-PMv; a to f and m to r, success rate for retrieval; g to l and s to x, prehension time; a, d, g, j, m, p, s, and v are preoperative trials; b, e, h, k, n, q, t, and w are trials at the early stage of recovery; c, f, i, l, o, r, u, and x are trials at the late stage of recovery. Error bars indicate standard deviation. $^{*}P < 0.05$, $^{**}P < 0.01$ (corrected t test). CS, central sulcus; Ipsi, ipsilesional hemisphere; Contra, contralesional hemisphere; Preop, preoperative trials; Early, trials during the early stage of recovery; Late, trials during the late stage of recovery; R, rostral; C, caudal; and N.A., not available.

stable control. Clarification of the neural pathways from these regions to the relevant motoneurons will lead to a deeper understanding of the strategy for functional compensation and, in addition, will provide a good indication of the prospects for recovery after spinal cord injury.

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Materials and Methods
Figs. S1 to S3
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Social Integration of Robots into Groups of Cockroaches to Control Self-Organized Choices

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Collective behavior based on self-organization has been shown in group-living animals from insects to vertebrates. These findings have stimulated engineers to investigate approaches for the coordination of autonomous multirobot systems based on self-organization. In this experimental study, we show collective decision-making by mixed groups of cockroaches and socially integrated autonomous robots, leading to shared shelter selection. Individuals, natural or artificial, are perceived as equivalent, and the collective decision emerges from nonlinear feedbacks based on local interactions. Even when in the minority, robots can modulate the collective decision-making process and produce a global pattern not observed in their absence. These results demonstrate the possibility of using intelligent autonomous devices to study and control self-organized behavioral patterns in group-living animals.

Self-organization is a central coordination mechanism exhibited by both natural and artificial collective systems. Collective behavior and decision-making based on self-organization occur in eusocial insects (*1*–*3*), gregarious arthropods (*4*, *5*), and vertebrates (*6*–*8*). Self-organized mechanisms are characterized by nonlinear responses to stimulus intensity, incomplete information, and randomness (*1*). Self-organization coexists with guidance from environmental templates, networks of interactions among individuals, and various forms of leadership or preexisting individual specialization (*9*, *10*). Studies of animal societies (*1*–*8*) show that self-organization is used to coordinate group members, to reach consensus, and to maintain social coherence when group members have to choose between mutually exclusive opportunities.

These biological findings have stimulated engineers to investigate novel approaches for the coordination of autonomous multirobot systems (*11*–*14*). Swarm-robotic systems, in contrast with other multirobot systems, explicitly exploit self-organization as a main coordination mechanism. Often, the controller of individual robots is designed using reactive, behavior-based techniques (*15*): Robots act and interact with their close environment, which sends immediate feedback to their receptors in response to their own actions and the actions of others. Behavior-based techniques allow for real-time implementation of the social nonlinear feedbacks influencing the whole system, minimization of onboard computational resources under tight volume constraints, and suitable support for the injection of stochastic behavioral rules.

Autonomous robots, perceived as congeners and acting as interactive decoys, are interesting research tools. By their ability to respond and adapt to animal behavior, they open possibilities to study individual and social animal behaviors. Robots, or any artificial agents, could then be used to implement new feedback loops, leading to new collective patterns in these mixed natural-artificial systems. Here we describe an experimental study that makes a step toward building such mixed societies of artificial and natural agents, using real and robotic cockroaches.

Our experimental setup consists of a circular arena endowed with two shelters (Fig. 1). In the presence of two identical shelters, each large enough to host the entire group, all the cockroaches choose collectively to rest under one of the shelters (*16*, *17*). When one shelter is darker than the other, cockroaches select the darker shelter by amplifying their individual preference through interindividual interactions. This self-organized choice does not require leadership, reference to the final pattern, or explicit comparison between the shelters. This mechanism leads to shelter selection and optimal group formation (*17*).

A mathematical model in quantitative agreement with the experiments was developed (*17*) considering the following experimental facts: (i) Individuals explore their environment randomly and thus encounter sites randomly; (ii) they rest in sites according to their quality, in this case determined mainly by darkness; and (iii) they are influenced by the presence of conspecifics through social amplification of resting time, all individuals being considered equal. This model also forms the core behavioral module of the robots, enabling them to respond stochastically to social stimuli according to Eqs. 1 to 4 (below). The robots are designed to discriminate (i) cockroaches from other robots, these two types of agents being considered here as conspecifics; (ii) shelters from the rest of the arena and shelter darkness; and (iii) the wall around the circular arena and other obstacles (*18*). The model is used as a quantitative explanation as well as overall guidance for the design of the robot.

The model describes mixed groups where robots and cockroaches exhibit similar behavior. The differential equations giving the time evolution of the number of individuals in the shelters and outside are

$$dx_i/d_t = R_i x_e - Q_i x_i \quad i = 1, 2 \qquad (1)$$

$$dr_i/d_t = R_{ri} r_e - Q_{ri} r_i \quad i = 1, 2 \qquad (2)$$

$$C = x_e + x_1 + x_2 \qquad (3)$$

$$M = r_e + r_1 + r_2 \qquad (4)$$

Variables x_i and r_i represent the numbers of cockroaches and robots present in shelter i, respectively, and x_e and r_e the numbers outside the shelters. Parameters C and M correspond respectively to the total numbers of cockroaches and robots. The functions R and Q, giving

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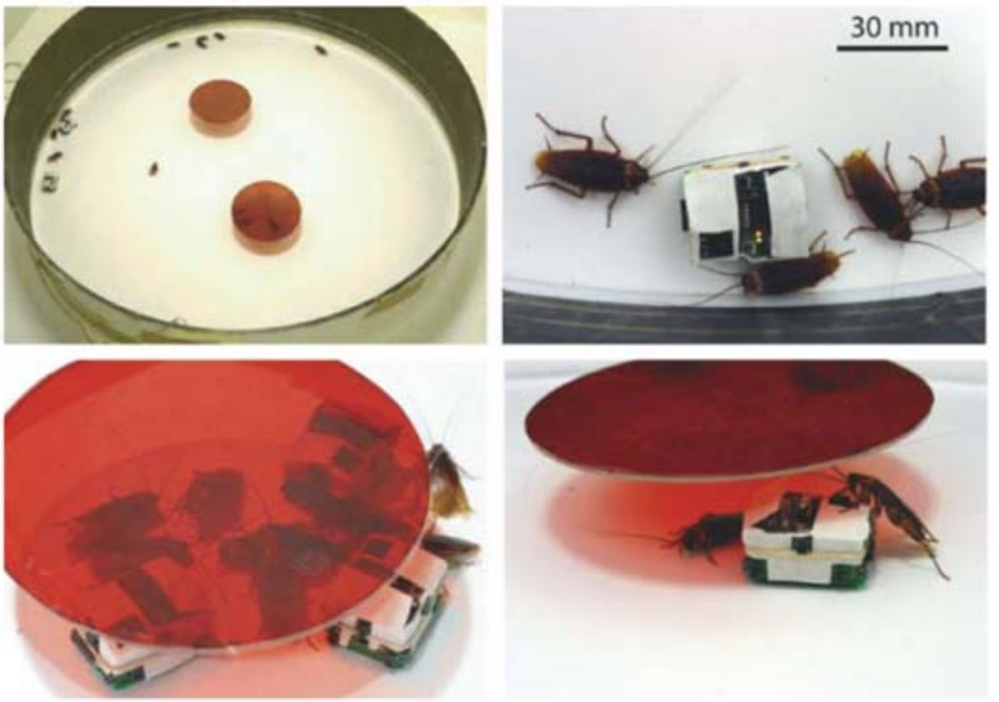

Fig. 1. Experimental setup showing the cockroaches (*Periplaneta americana*) and the robots. Two shelters (150 mm) made of plastic disks covered by red film filters are suspended (30 mm) above the floor of a circular arena (diameter 1 m). The darkness under the shelter is controlled by the number of layers of red film. Cockroaches aggregate under the shelters (*18*).

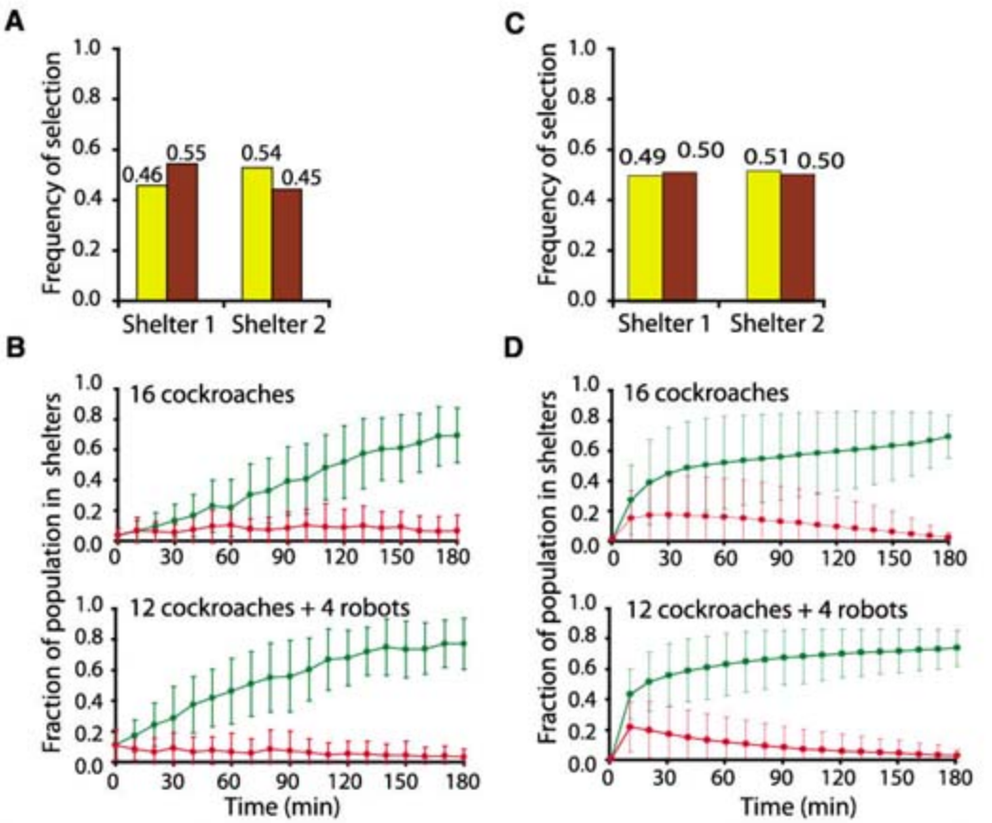

Fig. 2. Shared collective choice between two identical shelters. (**A** and **B**) Experimental results for 30 trials. (**C** and **D**) Computer simulations of Eqs. 3 and 4 (*18*). Groups of 16 cockroaches (brown bars) selected one of the two shelters. Mixed groups of 12 cockroaches and four robots (yellow bars) presented the same distribution, demonstrating that the mixed groups made the same collective decision as cockroaches alone. The probability of selecting one of the shelters is about 0.5, in accordance with a dynamics leading to stable multiple states (*16*, *17*). In (B) and (D), the fraction of the group present under the shelters (mean ± SD) in relation to time shows that selection has similar dynamics in both types of group. Green lines represent the selected shelter (randomly shelter 1 or 2 in different trials), the red lines the shelter not selected.

respectively the rate per individual of entering or quitting shelters, are

$$R_i = \mu_i\{1 - [(x_i + \omega r_i)/S_i]\} \quad (5)$$

$$R_{ri} = \mu_{ri}\{1 - [(x_i + \omega r_i)/S_i]\} \quad (6)$$

$$Q_i = \theta_i/\{1 + \rho[(x_i + \beta r_i)/S_i]^n\} \quad (7)$$

$$Q_{ri} = \theta_{ri}/\{1 + \rho_r[(\gamma x_i + \delta r_i)/S_i]^{n_r}\} \quad (8)$$

Each cockroach outside shelters has a rate R_i of entering shelter i (R = 1/mean exploring time); the equivalent rate for robots is R_{ri}. Because these functions (Eqs. 5 and 6) take into account a crowding effect, they decrease with the ratio between the number of individuals present in shelter i and its carrying capacity S_i. The carrying capacity corresponds to the maximum number of cockroaches that can be hosted in shelter i. In Eqs. 5 and 6, parameter ω represents the surface of one robot expressed as a multiple of the surface of one insect. The term μ_i represents the maximal kinetic constant of entering the shelter for insects; μ_{ri} is the equivalent term for robots.

Each cockroach in shelter i has a rate Q_i of leaving it to start exploring (Q = 1/mean resting time); the equivalent rate for robots is Q_{ri}. The parameter θ_i is the maximal rate of leaving a shelter for cockroaches (θ_{ri} for robots); the parameters ρ and n take into account the influence of the cockroaches' conspecifics (ρ_r and n_r for robots). When both shelters are identical, the parameters characterizing them are equal: $S_1 = S_2$; $\mu_1 = \mu_2$; $\mu_{r1} = \mu_{r2}$; $\theta_1 = \theta_2$; $\theta_{r1} = \theta_{r2}$. When one shelter is darker than the other, then $\theta_1 \neq \theta_2$; $\theta_{r1} \neq \theta_{r2}$.

Parameters γ, β, and δ correspond respectively to the influence of insects on robots, of robots on insects, and of robots on robots. The greater they are, the greater the mutual influences. The influence of insects on insects is imposed by biology and is not modulated in our experiments. However, parameters γ, δ, and β could be modulated by changing the hardware and/or software of the robots. As in insect societies, the interattraction between cockroaches is chemotactile and is mainly based on a blend of hydrocarbons coating their body (*19*–*22*). The robots are coated with this blend, and the higher the pheromone concentration, the higher the value of β.

Acceptance of robots within a cockroach group is related to the ability of robots to bear the correct chemical signal and to behave appropriately. Chemical analyses and behavioral tests were performed to identify the main molecules constituting the odor that carries cockroach identity (*18*). This odor was then collected from male cockroaches and calibrated to a known concentration used to condition filter papers dressing the robots. The concentration on the filter paper (per cm^2) was the same as that on one cockroach. Therefore, natural and artificial agents were equally attractive to one another. Tests with encounters between robots and cock-

roaches showed that cockroaches were lured to, and interacted with, chemically dressed robots. Comparisons with unmarked robots showed the importance of this chemical message (*18*).

Pheromone luring was used here to allow acceptance of the robot in the group and not to attract the insects to a specific shelter. As robots become members of the group, they can take part in and influence dynamically the collective decision-making process. Not only do these robots explore their environment autonomously, but they are also able to tune their resting time in relation to the presence of cockroaches, as cockroaches do (*16*, *17*). In turn, the insects are influenced by the presence of robots, closing the loop of interaction between animals and machines. The shelter selection emerges from the social interactions between natural and artificial individuals.

The first set of experiments showed the sharing of the collective decision-making for shelter selection in mixed cockroach-robot groups. The robots were programmed to select dark shelters as cockroaches do. Interactions between robots and cockroaches led to the selection of a common shelter (Fig. 2). Given the choice between two identical dark shelters, both types of groups chose to rest under one of the shelters and behaved as a whole, irrespective of their natural or human-made origin. In most trials, both cockroach groups and mixed groups selected one of the shelters. In 28 of 30 trials (93%), mixed groups presented a clear choice for one of the shelters, and 75% of cockroaches and 85% of robots aggregated under the same shelter. Comparisons of these results with computer simulations of the model confirmed that the choice corresponds to the coexisting stable states of a nonlinear system (Fig. 2, A and C).

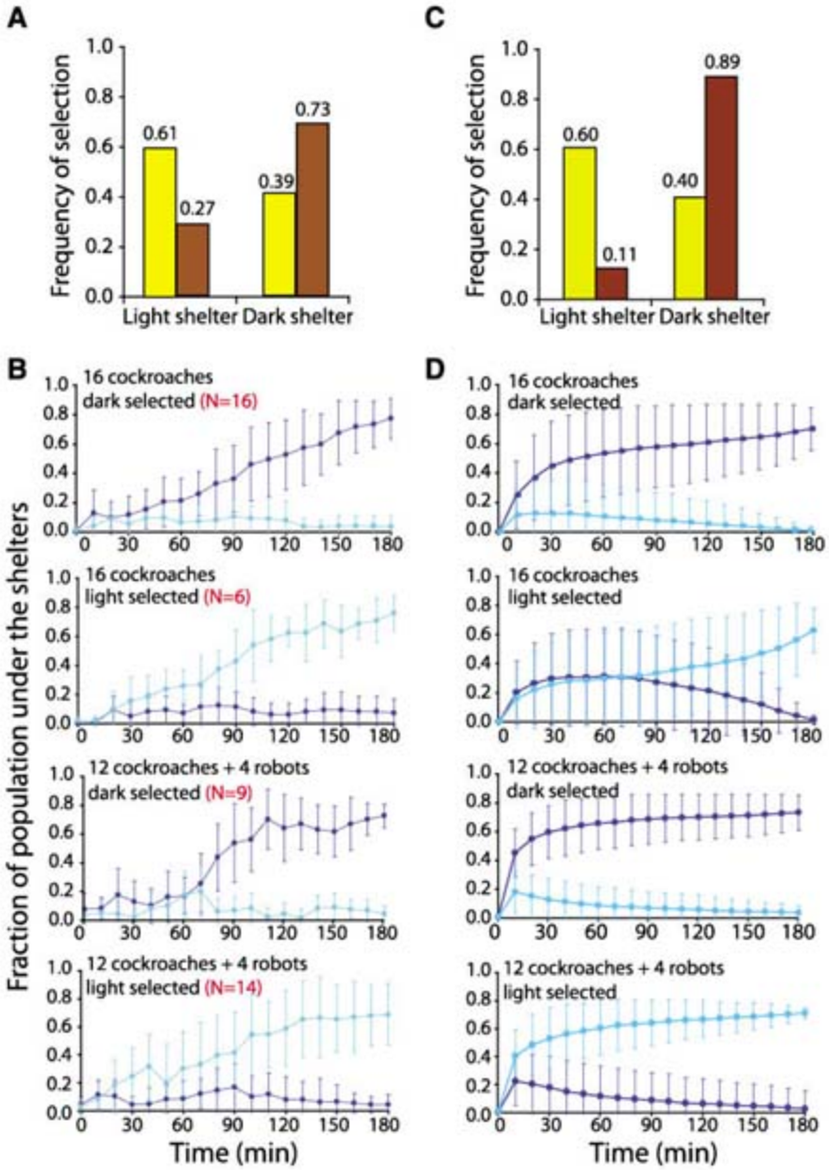

Fig. 3. Controlled collective choice between dark and light shelters. (**A** and **B**) Experimental results; (**C** and **D**) computer simulations (*18*). (A) Groups of cockroaches without robots (brown bars) selected the dark shelter in 73% and the light shelter in 27% of the trials. Mixed groups with robots programmed to prefer the light shelter (yellow bars) selected it in 61% of the trials. The robots induced a change of the collective choice by modulating the nonlinear collective mechanism. Nonetheless, the dark shelter was still selected in 39% of the trials because the robots also socially responded to the cockroaches. In all selections, robots and cockroaches shared the same shelter. In (B) and (D), the fraction of the group present under the shelters (mean ± SD) as a function of time shows that the selection has similar dynamics in both types of group (dark blue, dark shelter; light blue, light shelter). *N* values in (B) (red) are number of selections out of 30 trials.

The second set of experiments was designed to show the control of the collective choice by mixed groups when shelters differed in attractiveness—in this case, darkness (Fig. 3). Cockroaches prefer to aggregate under the darker shelter (brown bars in Fig. 3A). This selection process is explained by the same model as above, with a bias induced by the darkness level of the shelters ($\theta_1 \neq \theta_2$, $\theta_{r1} \neq \theta_{r2}$; Fig. 3C). When cockroach groups selected one of the shelters (22 of 30 trials), the darker shelter was selected in 73% of the cases and the lighter one in only 27% of the cases (Fig. 3A). As in the first set of experiments with two identical dark shelters, these proportions correspond to the coexistence of multiple stable states in a nonlinear system.

In the case of mixed groups (yellow bars in Fig. 3A), the robots were programmed to prefer the lighter shelter, contrary to the cockroaches. This effect was obtained by keeping the same behavioral model and swapping the parameters controlling the robot response to darkness with respect to those measured for cockroaches. Given the choice between a dark and a light shelter, robots were able to induce a change of the global pattern by inverting the collective shelter preference. Under these conditions, the shelter less preferred by the cockroaches (i.e., the lighter one) was selected by mixed groups in 61% of the trials, versus only 27% of the trials done without robots. Despite the individual preference of robots for lighter shelters, they were socially driven by the cockroaches into the darker shelter in 39% of the trials (Fig. 3A). These results are explained by the nonlinear mechanism governing the self-organized choice, as shown by stochastic simulation of the model (Fig. 3B). In some trials the choice was induced by the robots, and in others by the cockroaches. The robots did not act as a mere attractant but were integrated into the decision-making process of the society.

These experimental results show the possibility of shared and controlled collective actions between machines and animals. At the technical level, we introduced lures able to perceive animal response and able to respond to it. The robots were designed to interact and to collaborate autonomously both with the animals and with one another. This work could be extended to vertebrates, taking into account sound, visual cues, and social organization. Possible ways to identify individual behavioral algorithms could be to replace some animals within a group by robots or other artificial devices and to compare collective responses in "mixed" and "natural" groups (*23*–*26*). They could also be used to test

hypotheses about the origin of cooperation among group members. At the conceptual level, we exploited the nonlinear dynamical properties of regulatory feedbacks to introduce a form of control that can require only a small number of social force. Artificial agents such as robots or networks of sensors and actuators could also be used to introduce new regulatory feedback loops (or modulate existing ones) at the social level (*27*, *28*), inducing new patterns of collective behavior. Animal societies could be one of the first biological systems where autonomous artifacts cooperate with living individuals to solve problems.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5853/1155/DC1
Materials and Methods
Figs. S1 and S2
Tables S1 to S4
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Tamas Horvath, D.V.M., Ph.D.
Professor and Chair, Section of Comparative Medicine
Yale University School of Medicine
P.O. Box 208016
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E-mail: valeria.krizsan@yale.edu

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Tenured and Tenure-Track Faculty Positions in Systems Biology

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The successful applicant is expected to conduct a strong research program and to contribute to the teaching of undergraduate and graduate students. Systems biology research and training at UCI is fostered by several interdisciplinary research units, a National Insitute of General Medical Sciences National Center for Systems Biology, and Ph.D. training programs in bioinformatics, and mathematical and computational biology (for more information, see **website: http://ccbs.bio.uci.edu).** Applicants should submit a letter of application, curriculum vitae, bibliography, three letters of reference, and statements of research and teaching interests using the online recruitment system (see instructions at **website: http://ccbs.bio.uci.edu or https://recruit.ap.uci.edu).** To receive full consideration, material should be received by January 1, 2008.

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OPENING DOORS FOR SCIENTISTS WITH DISABILITIES

Several programs are aiming to increase the number of individuals with disabilities in science and technology careers by removing barriers and changing attitudes. **By Laura Bonetta**

Chad Cheetham is pursuing a Ph.D. in neuroscience at the University of Alabama in Birmingham. He is one of six students at his institute to have received a coveted Howard Hughes Medical Institute scholarship for his graduate work. **Megan Nix,** an electrical engineering graduate of the University of California, Riverside is looking for a full-time position, probably at the Jet Propulsion Laboratory (JPL) where she interned in the spring of 2005. She received first place in a competition from the Institute for Electrical and Electronics Engineers for her project at JPL.

These are typical success stories of students pursuing careers in scientific fields, except that the students happen to have a disability. Cheetham has no left visual cortex, which means he lacks the right visual field and depth perception, while Nix has fibromyalgia, a chronic condition that causes widespread pain in the body and exhaustion.

In many cases, disabilities are not barriers in science and technology fields, where mental capacity and creativity are keys to success. Nonetheless, individuals with disabilities face unique challenges as they transition from high school to college and from college to employment.

They might need software or other technologies to help them follow along in classes, face problems finding adequate living arrangements close to their university, or come up against faculty or employers who are fearful of dealing with a person with a disability. A number of programs and resources are helping to alleviate such challenges.

The Voice of Experience

Ted Conway did not divulge to prospective employers that he had cerebral palsy. When invited for an in-person interview, he would explain he had a loss of muscle action caused by a lack of oxygen during birth to the part of the brain that controls muscle movement. "I always describe what the disability does rather than calling it by its name," says Conway. "If people hear cerebral palsy, or muscular dystrophy, or cancer, they always think the worst."

A professor and associate dean at Virginia Commonwealth University, Conway has, for the past 21 years, been going up the academic ladder in the fields of mechanical, aerospace, and, more recently, biomedical engineering. He has held jobs in industry, government, academia, and as a consultant. "The only challenges that I have faced have been overcoming other people's predetermined ideas about what a person with a disability could do," he says.

An effective way for attitudes to change is for more people to see individuals with disabilities in established positions. "Role models serve as examples, but also act as mentors for people who want to acquire that position," says Conway. "Someone has to blaze that trail and then the next person who comes along can ask 'What do I have to do to get there?'"

Increasing Numbers

A handful of programs are trying to increase the numbers of individuals with disabilities in science, technology, engineering and math (STEM) fields. Eleven years ago, the American Association for the Advancement of Science (AAAS), publisher of the journal *Science,* established EntryPoint! The program provides internship opportunities to students with disabilities at IBM, Merck & Co., the National Oceanic and Atmospheric Administration (NOAA), the National Institute of Standards and Technology (NIST), Lockheed Martin, CVS, NAVAIR, and NASA.

continued »

> "The only challenges that I have faced have been overcoming other people's predetermined ideas about what a person with a disability could do."

From top: Intern **Matthew Maleski** in front of his poster at the Stanford/CPIMA/IBM program; **Chad Cheetham,** a neuroscience student at the University of Alabama, Birmingham; intern **Brittany Toffinchio; Aubrie Abbott** with Incight Scholar **Alison Ecker** at her summer 2007 internship site.

CREDIT: (TOP) CPIMA; (BOTTOM RIGHT) KEITH OZOLS

UPCOMING FEATURES

Interdisciplinary Research — November 23

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Diversity

EntryPoint! alumni: Royce James (left) interned at NASA Goddard and NSF. He is completing his physics Ph.D. and is the first African American tenured faculty at the US Coast Guard Academy. Jason Grieves interned at IBM and will graduate in 2008 from Virginia Tech.

"A persistent student can get an undergraduate degree. There are barriers, but if you want to do it, you can do it. It may be harder at the graduate level. But it is harder still to get employment in your field," says EntryPoint! Director **Virginia Stern.** "The internship is critical. The employer gets to know you and what you can do. And you find out what you want to do."

To participate in EntryPoint! a student with a disability not only has to be interested in STEM careers but also have a 3.0 or above grade point average. "The organizations we work with want the diversity, but they need competitive students," says Stern. "We do the talent search."

Cheetham spent a summer at Merck & Co. where he was in charge of developing an assay to screen compounds related to obesity. "EntryPoint! does not lower expectations. They only take the best," says Cheetham. "They are advocates for people with disabilities, but they want really qualified students. It's not 'Poor me give me an internship because you feel sorry for me.' It's 'Give me an internship because I am really good!'"

Successful work experiences are not only critical to opening career doors; they also change the attitudes of employers who may be wary of hiring individuals with disabilities. "We make sure that the employer has a positive experience," says **Sheryl Burgstahler,** director of the Disabilities, Opportunities, Internetworking and Technology (DO-IT) program at the University of Washington. "If there is a problem we intervene, and most of the time it is not a disability-related issue. That is what we help the employer see."

DO-IT, a multifaceted program to help people with disabilities succeed in college and the work force, includes an online mentoring network and an internship program that are part of the program entitled Access to Science Technology, Engineering and Mathematics (AccessSTEM). It provides about 50 internship placements a year in the states of Oregon, Washington, Alaska, and Idaho.

Enabling Technologies

Established in 1992, AccessSTEM makes extensive use of computers, assistive technologies, and the Internet to help students with disabilities become more independent in their academic and career activities. "An employer might say 'How can you have a blind person do programming?' But it is not hard. You need a standard computer with a refreshable braille display and a braille printer," explains Burgstahler. "We want to show that with the right technology people with disabilities can succeed."

Help obtaining those technologies can be a boon to students. "Most assistive technology is overpriced and yet may be a student's sole means of communication or may give someone the ability to use a computer," says **Chris Schlechty,** a senior at the University of Washington studying computer science.

Schlechty has limb girdle muscular dystrophy and uses a power wheelchair to get around. "I need an accessible workstation, which consists of a certain keyboard and mouse set, a height adjustable desk, and an alternate headset or handset for the phone as I cannot lift up the receiver," he explains.

Schlechty interned at Microsoft through the DO-IT program. After graduating in June 2008, he hopes to obtain employment at Microsoft or one of the other major software companies in the area. "A student should not prematurely label classes or careers as inaccessible. By working with the professors and using a bit of creativity, we were always able to make accommodations that worked, and I have been able to successfully complete all of my courses, including those that seemed to require a fair amount of physical activity," says Schlechty.

The National Science Foundation has supported DO-IT's AccessSTEM and other similar programs through its Research in Disabilities Education (RDE) program. Other RDE awards include projects that develop new assistive technologies for people with disabilities. One example, developed by a team at Pennsylvania State University, University Park, is a hand-held submersible audible light sensor that fits in a test tube and converts the light intensity to an audible signal to help blind scientists conduct chemistry experiments.

STEM Careers Make Sense

Individuals with disabilities are generally underrepresented in science and engineering professions. Nevertheless the employment rate for scientists and engineers with disabilities is 83 percent, much better than the estimated 26 percent for the overall US population with disabilities. These statistics suggest that the engineering and science fields provide careers in which individuals continued »

DO-IT (Disabilities, Opportunities, Internetworking, and Technology)
www.washington.edu/doit

EntryPoint!
www.ehrweb.aaas.org/entrypoint

IBM
www.ibm.com

Incight
www.incight.org

Jet Propulsion Laboratory (JPL)
www.jpl.nasa.gov

Lockheed Martin
www.lockheedmartin.com

Merck & Co.
www.merck.com

National Institute of Standards and Technology (NIST)
www.nist.gov

NOAA (National Oceanic and Atmospheric Administration)
www.noaa.gov

NSF's Research in Disabilities Education www.nsf.gov/pubs/2007/nsf07511/nsf07511.htm

Pennsylvania State University
www.psu.edu

Stanford University's Center on Polymer Interfaces and Macromolecular Assemblies (CPIMA)
www.stanford.edu/group/cpima/

University of Alabama
www.ua.edu

University of California, Riverside
www.ucr.edu

University of Oregon
www.uoregon.edu

Virginia Commonwealth University
www.vcu.edu

Additional Online Resources

Eastern Alliance in Science, Technology, Engineering & Mathematics
research.usm.maine.edu/East

Midwest Alliance in Science, Technology, Engineering & Mathematics
www.stemmidwest.org

Regional Alliance for Science, Engineering & Mathematics
rasem.nmsu.edu

"The response has been favorable. A number of interns have been repeat interns and a couple will be picked as permanent employees."
–Julie Peddy

with disabilities can find success.

"I actually think those fields are good ones for students with disabilities to get into, because there are just so many opportunities available to help get women, minorities, and now people with disabilities involved, since they are so underrepresented," says **Alison Ecker,** a junior at the University of Oregon majoring in comparative literature.

Ecker, who is hard of hearing, completed a DO-IT internship in viticulture, an area outside her field of study. Because of the internship, she would now consider a career in scientific research. "I would highly recommend having an internship, possibly even before deciding a major, as it allows you to get real-life experience, to see if it's a career that you might actually be interested in," she says.

Why are STEM careers a good match for individuals with disabilities who have an interest in these fields? "It is a combination of things. There tends to be an increased use of technology in those fields which makes it easier to integrate assistive technologies," says Burgstahler. "STEM jobs are often not physically demanding jobs. You are using your head, not your muscle."

The Employers' Perspective

And if STEM careers make sense for people with disabilities, it also makes sense for employers to hire them. "We are competing with countries that have plenty of individuals with technical expertise. We cannot afford to leave any talented people out of the work force," says **Ted Childs,** former vice president of global diversity at IBM.

Like IBM, the Center on Polymer Interfaces and Macromolecular Assemblies, an NSF-sponsored center and a joint effort between Stanford University and IBM Almaden Research Center, has had students with disabilities as summer interns for the past six years.

These internships required making some changes in the buildings, such as adding touch plates to doors, and making other accommodations, including hiring sign language interpreters during meetings and seminars as well as purchasing some special software. "It is a combination of changes in the buildings and working with the students to find out what they need," says center director **Curtis Frank,** who had two students with disabilities in his own lab.

But Frank sees many advantages to these internships. "For the other group members, it gives them an example of what can be accomplished. My group already has a good collegial working relationship. But having someone with special needs helps bring the group even closer together," says Frank. "It requires more folks to pay attention to what is happening in the lab."

Julie Peddy, program manager at NOAA's Northwest Fisheries Science Center and EntryPoint! coordinator for NOAA, has also had good experiences hosting students with disabilities as summer interns. "Some employers are worried about what the cost will be, but for the most part it is not costly to provide some accommodations for a person with a disability," she says. "The response has been very favorable. A number of interns have been repeat interns and a couple will be picked up as permanent employees."

Changing Attitudes

Many scientists with a disability, particularly one that is apparent, say it is important to discuss the disability with teachers and prospective employers and advocate for whatever accommodations are needed to succeed. "As a student you have to make sure that you are not excluded from obtaining the same skills, or equivalent skills, as everyone else in the class," says **Imke Durre,** a physical scientist at NOAA. "Part of that responsibility falls on the teacher, but it is also up to the student to say, 'This is how I could do it.'"

After completing her Ph.D. in atmospheric science from the University of Washington, Durre applied for a fellowship from the National Research Council. Durre, who is blind from birth, added a "personal statement" in her application explaining what accommodations she uses. "I wrote 'This is how I handle graphics. This is how I read print documents,' and so on," she explains. "The approach worked for me."

She landed a postdoctoral position at NOAA's National Climatic Data Center, which later converted to a staff position in the Climate Analysis Branch. Durre got hooked on climate science as a child, when her mother would read her the newspaper's weather page. It never occurred to her that this was something she could not do. "I did encounter a teacher in junior high school who did not think I could do higher-level math, but I did not pay much attention," she says. "I figured she did not know me."

Incight, a not-for-profit organization based in Portland, Oregon, works with high school and college students with disabilities to help them overcome their own fears and become better advocates for themselves. "When we hear 'I would like to do this but I don't think I can do it,' that is when we get really motivated," says Incight's **Aubrie Abbott.** "We work with them and say 'Well, actually, we think you can. Let's figure out the steps you need to get there.'"

Incight works closely with a set of college students from all over the country, providing them with scholarships, mentors, and assistance in finding internships. This year the scholarship program, which started only four years ago, received 800 applications for 70 spots. In addition, Incight helps prepare Oregon high school students for life after graduation, through training and mentoring. "By the time they get to college they are better at being their own advocates," says Abbott.

Programs like EntryPoint!, DO-IT, Incight, and many others are working to change the face of research by providing tools and advice to talented students who have disabilities. They are also creating networks of students and professionals with disabilities who can serve as role models for others to follow. "Eventually we would like to put ourselves out of business," laughs Abbott. "In a perfect world you would not need us. We are trying to develop leaders who can remove barriers and pave the way."

Laura Bonetta is a scientist turned freelance writer based in the Washington, D.C., area.

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UNCF • MERCK SCIENCE INITIATIVE

"A mind is a terrible thing to waste"

MERCK

UNDERGRADUATE SCIENCE RESEARCH SCHOLARSHIP AWARDS

- 15 Awards Annually
- Scholarships up to $25,000
- Two Summer Internships at a Merck Research Facility

An applicant must:

- Be a full-time student at any four-year college or university
- Have junior year academic status
- Major in a life or physical science (first professional degrees excluded)
- Have a minimum cumulative GPA of 3.3 (4.0 point scale)

GRADUATE SCIENCE RESEARCH DISSERTATION FELLOWSHIPS

- 12 Fellowships Annually
- Fellowship Stipends up to $42,000
- Department Grants of $10,000
- Support for 12-24 months

An applicant must:

- Be enrolled full-time in a Ph.D. or equivalent doctoral program in a biomedical life or physical science
- Be engaged in and within 1-3 years of completing dissertation research

POSTDOCTORAL SCIENCE RESEARCH FELLOWSHIPS

- 10 Fellowships Annually
- Fellowship Stipends up to $70,000
- Department Grants of $15,000
- Support for 12-24 months

An applicant must:

- Hold a Ph.D. or equivalent degree in a biomedical life or physical science
- Be appointed as a new or continuing postdoctoral fellow by the end of 2008 at an academic or non-academic research institution (private industrial laboratories are excluded)

Applicants must be African American (Black), U.S. citizens or permanent residents, and attending an institution in the U.S.A. Applications must be submitted online at www.uncf.org/merck/ by December 17, 2007

For more information, please contact your department chairperson or Jerry L. Bryant, Ph.D., at the **United Negro College Fund, Inc.**, 8260 Willow Oaks Corporate Drive, P.O. Box 10444, Fairfax, VA 22031-4511, by fax (703) 205-3574, or by e-mail at ***uncfmerck@uncf.org***.

NATIONAL RESEARCH COUNCIL

OF THE NATIONAL ACADEMIES

Research Associateship Program

Postdoctoral Research Awards
Senior Research Awards
Summer Faculty Fellowships
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offered for research at
US government laboratories

Opportunities for postdoctoral and senior research in all areas of science and engineering

- Awards for independent research at approximately 100 participating laboratory locations
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- Annual stipend $41,000 to $70,000 - higher for senior researchers
- Relocation, professional travel, health insurance
- Annual application deadlines Feb. 1, May 1, Aug. 1, Nov. 1

Detailed program information, including instructions on how to apply, is available on the NRC Web site at :

www.national-academies.org/rap

Questions should be directed to :

National Research Council
TEL: (202) 334-2760
E-MAIL: rap@nas.edu

Qualified applicants will be reviewed without regard to race, religion, color, age, sex or national origin.

THE NATIONAL ACADEMIES
Advisers to the Nation on Science, Engineering, and Medicine

Department of Geosciences PRINCETON UNIVERSITY

The Department of Geosciences at Princeton University is seeking applications for a **tenure-track faculty position** in **solid-earth geosciences**. We are particularly interested in outstanding scientists who study Earth's lithosphere, mantle, or core, by geophysical (seismology, geodynamics, or mineral physics), geochemical, and/or geological (petrology, geochronology, or geomorphology) methods.

Applicants should send a curriculum vitae, including a publication list, a statement of research and teaching interests, and contact information for three references to: **Allan Rubin, Search Committee Chair, Department of Geosciences, Guyot Hall, Princeton University, Princeton, NJ 08544**. The starting date is flexible. Evaluation of applications will begin immediately; interviews of candidates will begin in the Fall of 2007 and will continue until the position is filled.

Princeton University is an equal opportunity employer and complies with applicable EEO and Affirmative Action regulations. For general information about applying to Princeton and how to self-identify, please link to http://web.princeton.edu/sites/dof/ApplicantsInfo.htm.

DIVERSITY

Department of Health and Human Services
National Institutes of Health
National Cancer Institute

Cancer Proteomics Technology Projects Manager

The Office of Technology and Industrial Relations (OTIR) of the National Cancer Institute (NCI), National Institutes of Health (NIH), Department of Health and Human Services (DHHS), invites applications for a Cancer Proteomics Projects Manager, GS-401/601-13/14. OTIR is currently coordinating program and research activities of the NCI Cancer Proteomic Technologies for Cancer Initiative - a large multidisciplinary initiative (**http://proteomics.cancer.gov**), to optimize, develop and apply proteomic technologies and data resources to solve mission-critical problems in cancer research.

The successful candidate serves as a Cancer Proteomics Technology Projects Manager assisting in efforts that integrate proteomic & genomic-based technology programs, metrology, and quantitative analysis in addition to cancer treatment and diagnostic programs since these technologies hold the promise to accelerate the discovery and development of diagnostics and drugs suitable for personalized therapy. Pertinent also to this position is interaction with OTIR/NCI's Innovative Molecular Analysis Technologies Program and Alliance for Nanotechnology in Cancer. This is a key management position for providing leadership, management, and oversight of a wide range of projects within the NCI Cancer Proteomic Technologies for Cancer Initiative. The individual selected for this position will be conversant with a range of biomedical and proteomics technologies and applications as they pertain to cancer diagnostics, prognostics, and treatment. In addition, a publication record in scientific and technology areas in biomedical research related to the measurement of clinically relevant peptides and proteins including genetics, genomics, biochemistry, cell signaling and protein chemistry is needed. Candidate needs to have proven experience in managing research programs and ability to assess technological 'big picture'. Excellent communication skills are required; having industrial experience is a plus. A Ph.D. in physical, chemical or biological sciences is desired, but not required.

Salary is commensurate with experience and a full Civil Service package of benefits (including retirement, health, life and long-term care insurance, Thrift Savings Plan participation, etc.) is available. The NCI vacancy announcement for these positions contains complete application procedures and lists all mandatory information which you must submit with your application. To obtain the vacancy announcements for this position, see announcement number **NCI-08-228790 DE/MP** at **http://usajobs.opm.gov**. Applications must be received by no later than **November 20, 2007**.

The National Institute of General Medical Sciences (NIGMS) in Bethesda, Maryland is seeking applications from outstanding candidates with a strong background in biochemistry for one Health Scientist Administrator position in the Division of Pharmacology, Physiology and Biological Chemistry, which supports primarily basic, non-disease-oriented research and training, including a substantial portfolio of research in biochemistry.

The incumbent for this position will be responsible for developing and managing a portfolio of research grants that support studies in redox biochemistry, bioenergetics, and metallobiochemistry and/or related areas. The ideal candidate will have a substantial background in one or more of these areas as well as a thorough grounding in such areas as organic chemistry, pharmacology or molecular biology. Prior experience in research at the molecular level is highly desirable.

Applicants must possess a Ph.D. or M.D. plus scientific knowledge and demonstrated expertise in biochemistry and at least one of the following areas: organic chemistry, molecular biology, pharmacology, or related areas, and knowledge of the NIH peer review and grants process. Salary is commensurate with qualifications, and includes a full package of benefits. A detailed vacancy announcement (**NIGMS-08-229223-DE & NIGMS-08-229223-MP**) with the mandatory qualifications and application procedures can be obtained via the NIGMS web page at **http://www.nigms.nih.gov/about/job_vacancies.html** and the NIH Home page at **http://www.jobs.nih.gov**. Questions on application procedures may be addressed to **Jennifer Gilman** at **(301) 594-2256**. Applications must be received by close of business **December 18, 2007**.

The National Institutes of Health inspires public confidence in our science by maintaining high ethical principles. NIH employees are subject to Federal government-wide regulations and statutes as well as agency-specific regulations described at **http://ethics.od.nih.gov**. We encourage you to review this information.

CANCER BIOLOGY
TENURE TRACK POSITION
LABORATORY OF CANCER BIOLOGY AND GENETICS

The Laboratory of Cancer Biology and Genetics (LCBG), Center for Cancer Research (CCR) at the National Cancer Institute (NCI), National Institutes of Health (NIH) with proven strength in cancer genetics, signaling, chemical carcinogenesis, metastasis, molecular pharmacology, TGF-β biology, prevention and mouse models of human cancer, has recently undergone expansion (http://ccr.cancer.gov/labs/lab.asp?labid=825). As part of this growth, the LCBG now invites applications for a tenure track investigator to develop an independent research program in critical areas of cancer biology. The applicant should hold a Ph.D. and/or M.D. degree, and should have at least 3 years of postdoctoral experience; a substantive record of publications in quality peer-reviewed journals; and the potential to develop an outstanding independent program in basic and translational cancer research.

Salary is commensurate with education and experience. A one to two-page summary of research interests and goals should be submitted in addition to three letters of recommendation and a curriculum vitae postmarked by **December 20, 2007 to Ms. Patricia Martone, Executive Secretary, LCBG, CCR, NCI, Building 37, Room 4068, Bethesda, MD 20892-4255; Phone 301-496-3430; Fax 301-496-8709; E-mail: martonep@mail.nih.gov**.

Eligibility: Appointees must be U.S. citizens, resident aliens, or nonresident aliens with a valid employment-authorized visa. NIH tenure track investigators with educational debts may be eligible for the NIH Loan Repayment Program.

WWW.NIH.GOV

NIMH
National Institute of Mental Health

Tenured/Tenure Track Investigator

The Laboratory of Neuropsychology (LN) of the National Institute of Mental Health (NIMH) seeks a highly accomplished Tenured or Tenure Track Investigator conducting cognitive neuroscience research in nonhuman primates to head an active, ongoing program in this area. The position comes with a budget and staff. The strong scientific environment and outstanding resources at NIMH for carrying out such a program make this a unique opportunity for a high-achieving cognitive neuroscientist. The position also offers unparalleled opportunities for interdisciplinary collaboration with scientists throughout the NIH. The successful candidate will be expected to strengthen the current program.

Applicants should have: a Ph.D. and/or M.D. degree; experience as an investigator applying the techniques of neuropsychology, functional neuroanatomy, behavioral neuroimaging, behavioral electrophysiology, and/or neuropsychopharmacology to research with nonhuman primates; national/international recognition for studies in one or more of these disciplines; and experience in administering a neuroscience research program.

Salary is commensurate with experience and accomplishments, and a full Civil Service package of benefits (including retirement, health, life, and long-term care insurance, as well as a Thrift Savings Plan, etc.) is available. NIMH is a major research component of the National Institutes of Health and the Department of Health and Human Services, which have nationwide responsibility for improving the health and well being of all Americans. Interested applicants should send curriculum vitae, bibliography, statement of research interests, accomplishments, and goals, together with six letters of reference to: **Leslie Ungerleider, Chair, Search Committee for LN, NIMH, NIH, 10 Center Drive - MSC1366, Bldg. 10, Rm. 4C104, Bethesda, MD 20892-1366**; or email to **ungerlel@mail.nih.gov**. Application deadline: **January 2, 2008**.

NIEHS
National Institute of Environmental Health Sciences
National Institutes of Health

Senior Investigator, Tenure-Track Investigator, and Staff Scientist in Bioinformatics/Computational Biology – Biostatistics Branch

Research Triangle Park, North Carolina

The Biostatistics Branch is seeking scientists in Bioinformatics/Computational Biology. Candidates will be considered for Senior Investigator, Tenure-Track Investigator or Staff Scientist, depending upon qualifications (unless they explicitly specify a particular type of appointment).

As a **Senior Investigator**, the incumbent will develop and direct a strong research program including both investigator-initiated and collaborative research in the general area of bioinformatics and computational biology, particularly as related to biological networks, analysis of high-dimensional data, proteomics, comparative and functional genomics, gene expression, statistical genetics, and epigenetics. Responsibilities would also include assembling and managing a bioinformatics team to provide bioinformatics infrastructure and innovative data analysis approaches in support of intramural research aimed at understanding biological responses to environmental stressors in the context of cell biology, animal toxicology, clinical research and epidemiology.

The **Senior Investigator** will provide leadership in bioinformatics to new staff and to existing staff, consisting of a staff scientist and tenure-track investigator. Ideal candidates will have a proven history of individual and collaborative research excellence and an international reputation in a specific area within the broad general of bioinformatics and computational biology, with strong focus on biological applications.

Tenure-Track Investigator should have a strong publication record and robust and original research plans in the general area of bioinformatics noted above.

The **Staff Scientist** will work with the National Toxicology Program to assist in the identification of genes governing biological responses to environmental exposures, the evaluation and interpretation of biological response data from high-throughput toxicity screening assays, and the analysis of gene expression data from microarray studies. The ideal applicant will have experience in both bioinformatics and statistical genetics methodology.

All applicants must possess a Ph.D. or equivalent. Salary is commensurate with experience and level of accomplishments. Applications from women and members of minority groups are particularly welcome. To apply, submit a curriculum vitae, bibliography, brief statement of research interests and arrange for three letters of recommendation to the address below by **January 18, 2008**. Applications received after that date will be considered as needed.

Ms. Barbara Curtis (DIR07-06)
National Institutes of Health
National Institute of Environmental Health Sciences
P.O. Box 12233, Maildrop A2-06
111 Alexander Drive, Room A235
Research Triangle Park, NC 27709
email: dir-appls@niehs.nih.gov

DHHS and NIH are Equal Opportunity Employers

BCM

Baylor College of Medicine

Faculty Positions in Virology and Microbiology

The Department of Molecular Virology and Microbiology at Baylor College of Medicine invites applications for three tenure/tenure-track faculty positions, with rank commensurate with qualifications. Successful candidates will have demonstrated research productivity and will be expected to maintain an independent, innovative, funded research program and to participate in graduate training. This is an opportunity to join a strong, interactive department at Baylor College of Medicine in the rich scientific setting of the renowned Texas Medical Center. Current research interests of our departmental faculty focus on viral and bacterial gene expression and pathogenesis, RNA and DNA viruses of human diseases, emerging infectious diseases, vaccine development and evaluation, viral oncology, microbial genomics and proteomics, and antibiotic resistance. Outstanding core facilities are available, including BSL-2 and new BSL-3/3E containment facilities for handling infectious agents and infected small animals. Multidisciplinary research centers, including the Dan L. Duncan Cancer Center and the Center for AIDS Research, facilitate collaborations. Candidates in the following areas are invited to apply:

- **Influenza/Emerging Viral Infections** — Applicants with an interest in influenza viruses or other emerging viral infections are sought. Applicants with expertise in host–pathogen interactions, viral replication, and host responses to infection are encouraged to apply. Email: **BCM-MVM-facultypos1@bcm.edu**.
- **Bacterial Pathogenesis** — Applicants with an interest in Category A–C biodefense and emerging infectious disease bacterial pathogens and expertise in host–pathogen interactions, microbial genomics and proteomics, and host responses to infection are particularly encouraged to apply. Email: **BCM-MVM-facultypos2@bcm.edu**.
- **HIV/AIDS Research** — Applicants with expertise in HIV pathogenesis, HIV-related immunology, and AIDS-related malignancies are encouraged to apply. Email: **BCM-MVM-facultypos3@bcm.edu**.

Applicants should submit a curriculum vitae, a statement of research experience, a summary of future plans, and names of three references by **January 4, 2008** to: **Dr. Janet S. Butel, Faculty Recruitment Committee, Department of Molecular Virology and Microbiology, Mail Stop: BCM385, Baylor College of Medicine, Houston, TX 77030**. E-submission preferred using the email address listed for each position.

Baylor College of Medicine is an Equal Opportunity, Affirmative Action and Equal Access Employer.

WESTERN UNIVERSITY OF HEALTH SCIENCES
COLLEGE OF OSTEOPATHIC MEDICINE OF THE PACIFIC

FACULTY POSITIONS AVAILABLE FOR 2008-2009

Western University of Health Sciences is a thriving center for health care and veterinary education. Western University **www.westernu.edu** is headquarters to five colleges – Allied Health, Graduate Nursing, Osteopathic Medicine, Pharmacy and Veterinary Medicine. The University's core values have propelled the University to impeccable levels of excellence.

The University values a diverse institutional community and is committed to unparalleled excellence in its faculty, staff and students. Western University seeks applicants of distinguished academic and administrative accomplishments who possess a passion for excellence and can illustrate a proven track record of achievements.

The University is embarking on a new journey, adding another four graduate colleges at the same time by 2009 – Dentistry, Optometry, Podiatry and the Graduate College of Biomedical Sciences.

The Department of Basic Medical Sciences in the College of Osteopathic Medicine of the Pacific will provide the basis of the preclinical education and will require a significant increase in highly motivated, innovative and committed faculty members. A full range of tenure-track faculty positions with dual appointments to the above colleges at the level of Assistant, Associate or Full Professor are available. The successful applicant must have a Ph.D. or equivalent, and at least 2 years of postdoctoral experience. Applicants with demonstrated evidence of excellence in teaching and a commitment to inter-professional education will be given preference. Significant scholarly activity, publications and a strong potential to obtain extramural funding is expected. These positions will be available in the fall of 2008: **• Clinical Pharmacology (emphasis in anesthesia) • Endocrine Physiology • Genetics • Dental Histology • Histology • Human Anatomy • Oral Microbiology • Virology • Visual Neurophysiology.**

Applicants must submit the following information: (1) a current curriculum vitae; (2) a cover letter explaining how the applicant's background meets the requirements of the position applied for. This letter must include a brief statement including examples of teaching experience, philosophy, goals and research activity. Please include contact information for three references; (3) a completed Employment Application found at **http://www.westernu.edu/bin/hr/pdf/application_for_employment.pdf**. Send to: **Nissar A. Darmani, Ph.D., Chair, Department of Basic Medical Sciences, Western University of Health Sciences, College of Osteopathic Medicine of the Pacific, 309 E. Second Street, Pomona, CA 91766-1854; Email Address: ndarmani@westernu.edu.** Positions are open until filled.

Western University of Health Sciences is an Equal Opportunity Employer.

CHAVI

CENTER FOR HIV/AIDS VACCINE IMMUNOLOGY

POST DOCTORAL POSITIONS

The Center for HIV/AIDS Vaccine Immunology (CHAVI), occupying a place of national and international leadership in the fight against HIV/AIDS, is currently seeking candidates for two postdoctoral positions in our CHAVI RNA expression program. One position will work with Dr. Norman Letvin at Harvard Medical School, Beth Israel Deaconess Medical Center in Boston, MA and the other position will work with the CHAVI Director, Dr. Barton Haynes, and Dr. David Goldstein at Duke University and the Duke Institute for Genome Sciences and Policy in Durham, NC.

The overall goals of the CHAVI are to discover the enabling technology to produce a HIV/AIDS vaccine. The RNA expression program utilizing bioinformatics will help the CHAVI team learn more about the viral and immunological events and host genetic factors associated with HIV transmission and infection. Since its inception in July 2005, the CHAVI has established clinical trials sites in Africa, the United States and the United Kingdom, initiated the study of innate and adaptive cellular and antibody responses in acute HIV infection and exposed and uninfected patients, and established the EuroCHAVI Genetics Consortium. This is a multi-institutional team performing experimental and computational studies to test new vaccine strategies to overcome key immunological roadblocks in HIV vaccine design.

Qualified candidates should have a relevant Ph.D. and experience in RNA expression work or computational biology evaluating genomic data.

Please send a cover letter, current CV, and three letters of reference to the applicable location. If interested in either location, please indicate in your cover letter and send to: **Dr. Norman Letvin, Harvard Medical School, Beth Israel Deaconess Medical Center, 330 Brookline Ave., RE 113, Boston, MA 02215. Email: nletvin@bidmc.harvard.edu**

AND/OR

Dr. David Goldstein, Duke University Medical Center, Institute for Genome Sciences, 103 Research Dr., Box 3471, Durham, NC 27710. Email: dgoldstein@duke.edu.

CHAVI is an Equal Opportunity/ Affirmative Action Employer.

IMMUNOLOGY FACULTY POSITIONS
Roswell Park Cancer Institute

Under the leadership of a newly appointed department chair, Roswell Park Cancer Institute is committed to the expansion of its Immunology program. The Department of Immunology invites applications for positions equivalent to the Assistant, Associate and Full Member (Professor). Candidates are sought with a strong background in Immunology with special interest in Tumor Immunology, to complement and broaden the expertise of existing faculty in the department and strengthen the mission of the Institute as an NCI-designated Comprehensive Cancer Center.

Selection will be based on excellence in research and potential to maintain an outstanding independent research program. Applicants at the Associate and Full Member level will be expected to have current peer-reviewed funding and to maintain a productive research program. The new recruits will have the opportunity to contribute to the graduate education program at the Institute and to administrative responsibilities of the department. The Tumor Immunology Program has just successfully renewed its NCI predoctoral training grant (T32). We encourage applicants who desire an environment that fosters interaction with a diverse group of scientists and clinicians both within the Institute and the State University of New York at Buffalo.

Laboratory space in a newly opened 300,000 sq ft. Buffalo Life Sciences Complex provides a highly multidisciplinary environment, access to state-of-the-art core facilities, and the opportunity to interact with both research scientists and research-oriented clinicians from several departments.

Applicants should send their curriculum vitae, description of research accomplishments and of future research objectives and the names and addresses of three references to: **Dr. Yasmin Thanavala, Search Committee Chair, Department of Immunology, Roswell Park Cancer Institute, Elm & Carlton Streets, Buffalo, NY 14263**.

RPCI is an M/F/D/V Affirmative Action Employer.

ROSWELL PARK CANCER INSTITUTE

MAX PLANCK INSTITUTE FOR HUMAN COGNITIVE AND BRAIN SCIENCES LEIPZIG

In the Department **"Cognitive Neurology" (Director: Prof. Arno Villringer)** at the Max Planck Institute for Human Cognitive and Brain Sciences Leipzig three positions are available for

Junior Group Leaders

in the fields of "EEG-fMRI", "Data analysis of EEG/MEG signals" and "Human Cortical Plasticity". The main research areas of the department are "neurophysiology of neuroimaging signals in humans" and "plasticity after stroke".

The successful candidate holds a PhD or MD degree in a relevant research area and should have previous experience in one of the above mentioned research fields.

The Max Planck Institute offers a friendly and generous environment as well as excellent infrastructure (including human 3T and 7T MRI, EEG, TMS, NIRS, MEG). It brings together a large number of researchers with diverse backgrounds, united by their interest in the human brain and the methods for its exploration.

In order to increase the proportion of female staff members, applications from female scientists are particularly encouraged. Disabled applicants are preferred if qualification is equal.

For more information you may contact Arno Villringer (villringer@cbs.mpg.de).

The selection procedure starts as the applications come in. Please send your application (including CV, publication list, letter of motivation), citing the **code number "JGL 07"** to:

Max-Planck-Institut für Kognitions- und Neurowissenschaften - Verwaltung - Stephanstraße 1 a, D-04103 Leipzig www.cbs.mpg.de

MAX-PLANCK-GESELLSCHAFT

FALL 2008 Ph.D. PROGRAMS

THE SCRIPPS RESEARCH INSTITUTE

DOCTORAL PROGRAMS IN THE CHEMICAL AND BIOLOGICAL SCIENCES

The Kellogg School of Science and Technology at Scripps Research Institute will admit highly qualified chemistry and biology students to the La Jolla, California or the Jupiter, Florida campus to study biology, biophysics, chemical biology or chemistry, employing a highly interdisciplinary approach including a customized curriculum. The application deadline for Fall 2008 is January 1, 2008.

Established in 1961, Scripps Research Institute has gained international recognition for basic research in chemistry, structural, molecular and cell biology. Graduate studies at Scripps Research Institute provide an exceptional training opportunity in a uniquely multidisciplinary environment with emphasis on individualized training.

Candidates must have a bachelors degree and a strong background in biology, biophysics, chemistry, or a related discipline. Qualified applicants will be invited to visit the campus of admission. Financial support will be provided to all students accepted into the program.

Individuals interested in applying should visit Scripps Research Institute web sites: www.scripps.edu or www.scripps.edu/florida or contact::

Kellogg School of Science and Technology
The Scripps Research Institute
10550 N. Torrey Pines Rd. (TPC 19)
La Jolla, CA 92037

Tel: 858-784-8469 email: gradprgm@scripps.edu

TSRI is accredited by the Western Association of Schools and Colleges, 985 Atlantic Avenue, Alameda, CA 94501 (510-748-9001). Scripps Research does not discriminate on the basis of race, color, national or ethnic origin.

KELLOGG SCHOOL of science and technology

PROTEOMICS POSITION
UNIVERSITY OF WASHINGTON

The Department of Genome Sciences at the University of Washington School of Medicine in Seattle invites applications for one or more faculty positions at the rank of **ASSISTANT PROFESSOR** working in the field of proteomics on protein composition, function, dynamics, localization and interactions. Exceptional candidates may be considered at the rank of **ASSOCIATE** or **FULL PROFESSOR**.

Primary emphasis is on the establishment of an outstanding independent research program in proteomics, as well as participation in the teaching and service responsibilities with the Department of Genome Sciences. The position also provides association with the Proteomics Resource, which provides access to state-of-the-art equipment and facilities, as well as opportunities to establish collaborations with laboratories throughout the University of Washington and to play a leadership role in the University's wider proteomics program.

The Proteomics Resource facility occupies almost 7000 sq. ft. of space on the School's new Lake Union Campus and has obtained substantial long-term financial support of its mission, allowing for equipment acquisition and renewal, development of computing resources and recruitment of key personnel.

Applications received by **December 15, 2007**, will receive consideration. Thereafter, applications will be reviewed upon receipt until the position(s) are filled. Applicants should hold a Ph.D. or M.D. degree. Candidates should email their curriculum vitae, statement of research and teaching interests, and arrange to have three signed letters of reference sent to: **faculty-search@gs.washington.edu**. The Department of Genome Sciences web site is **http://www.gs.washington.edu**. The Proteomics Resource web site is **http://proteomicsresource.washington.edu**.

The University of Washington is building a culturally diverse faculty and strongly encourages applications from women and minority candidates. The University of Washington is an Affirmative Action/ Equal Opportunity Employer.

DUKE NUS
GRADUATE MEDICAL SCHOOL SINGAPORE

DIRECTOR and Senior Faculty
Program in Cardiovascular and Metabolic Diseases

The Duke-NUS Graduate Medical School Singapore (Duke-NUS GMS) is recruiting a **Program Director** to lead our signature program in Cardiovascular and Metabolic Diseases, with a primary focus on the prevention and treatment of vascular disease, including diabetes and obesity. The successful candidate will be a visionary scientist with an MD and/or PhD and an international reputation in either basic or clinical research. Also required is a demonstrated ability to establish and lead a multidisciplinary team of tenure-track faculty and associated junior faculty and trainees. The Program will be based in Singapore; however some research is likely to be conducted in countries in the region. In addition, we also seek **senior faculty** who have significant research accomplishments and a willingness to participate in a research enterprise that embraces a virtual academic environment that will reshape both fundamental views of cardiovascular disease and the delivery of effective diagnostic and therapeutic technologies. The positions will include full salary, generous start-up support, and five years of annual research funding.

The faculty of Duke-NUS GMS will have access to the world-renowned Duke Databank for Cardiovascular Diseases and the Duke Clinical Research Institutes databases and clinical trials portfolio. The Director should have a commitment to developing an integrated portfolio of research activities between the Duke-NUS GMS and Duke University in Durham NC.

Duke–NUS GMS is unique in bringing post-baccalaureate, research-intensive medical education to Asia, and represents a truly global partnership between two leading U.S. and Asian universities. Duke-NUS GMS shares a modern campus with Singapore's largest hospital and several national research centers, including the National Heart Centre. The facilities in nearby Biopolis, and those in the planning stages for proof of concept human studies and a clinical research coordinating center, will provide a unique array of complementary resources.

Interested candidates should email a cover letter stating the position applied for, curriculum vitae and a summary of research accomplishments to the Search Committee on Cardiovascular and Metabolic Diseases, Duke-NUS GMS: **CVMD.recruit@gms.edu.sg**

Igniting the Pioneer Spirit

Faculty Positions
Department of Pharmacology
The University of Michigan

The University of Michigan, Department of Pharmacology is seeking outstanding scientists for a tenure-track **ASSISTANT PROFESSOR** position. We are especially looking for an outstanding scholar with exceptional potential to develop a vibrant research program that augments current department initiatives in *Pharmacogenetics/genomics*, *Drug Metabolism*, or *Signal Transduction* (see **http://sitemaker.umich.edu/pharmacology/faculty_listing** for faculty interests). Qualifications include a Ph.D. in Pharmacology or a related discipline and/or M.D. degree, 3-5 years of postdoctoral experience, and research accomplishments as evidenced by scholarly contributions to the literature.

The successful candidate will join a dynamic, diverse, and collaborative department in a Top 10 Medical School in a university setting with superb opportunities for career development. The quality of life in Ann Arbor is outstanding. The combination of a large, major research university and a small, safe, family-oriented community make Ann Arbor an ideal environment to develop an academic and research career. Ann Arbor also offers an outstanding combination of sports, recreation, and cultural events.

Faculty members are expected to establish a highly visible externally funded research program and to excel in teaching medical students and other health professionals, as well as graduate and postdoctoral students. An attractive startup package including excellent laboratory facilities and generous startup funds will be available.

Send your CV, a two- to four-page summary of your research and future plans, and details of your teaching experience. Three letters of recommendation should also be sent. Address all correspondence to: **Chair, Pharmacology Search Committee, Department of Pharmacology, University of Michigan Medical School, 2301 MSRB III, 1150 West Medical Center Dr., Ann Arbor, MI 48109-5632**. Application materials may also be sent electronically to: **pharmsearch@umich.edu**.

The University of Michigan is an Affirmative Action/Equal Opportunity Employer. Applications from qualified women, minorities and/or disabled individuals are encouraged.

THE CHINESE UNIVERSITY OF HONG KONG

Applications are invited for:-

Department of Biology / Department of Computer Science and Engineering

(1) Professor(s) / Associate Professor(s) / Assistant Professor(s)

(Ref. 07/247(665)/2) (Closing date: February 29, 2008)

Applications are invited for two new faculty positions in bioinformatics and computational biology. Appointments will be made at Professor, Associate Professor or Assistant Professor levels as appropriate.

Applicants should have (i) a PhD degree in a relevant discipline, with preferably at least one year's postdoctoral experience; and (ii) strong commitment to excellence in teaching at undergraduate and postgraduate levels. Level of appointment will be commensurate with experience and qualifications. The appointees will work closely with the current team towards the formation of the centre/programme of bio-informatics and bio-technologies. The appointees will be a member of the Department of Biology or the Department of Computer Science and Engineering according to his/her area of expertise, and will join various campus-based centres that connect theoretical and experimental researchers in bioinformatics from different departments in biological, physical, mathematical and medical sciences, and engineering. Duties include (a) developing state-of-the-art research on integrative data analysis and interpretation using mathematical and statistical models for biological systems; (b) initiating and strengthening multidisciplinary collaboration addressing fundamental biological questions in model and non-model organisms; (c) conducting research with primary emphasis covering (but not limiting to) bioinformatics application of database, data mining, machine learning and algorithms; network modeling and systems biology; comparative genomics; or computational chemical genomics and structural bioinformatics; (d) establishing and maintaining a vigorous, innovative and collaborative research programme; (e) participating in teaching departmental and interdepartmental postgraduate programmes; and (f) developing and delivering courses in bioinformatics for students in related departments. Appointments will normally be made on contract basis for up to three years initially, leading to longer-term appointment or substantiation later subject to performance and mutual agreement. [Note: Those who have responded to the previous advertisement for this post (under Ref. no. 06/215/2) need not re-apply on this occasion.]

Department of Anatomy

(2) Research Assistant Professor

(Ref. 07/245(665)/2) (Closing date: November 19, 2007)

Applicants should have (i) a PhD or an MD degree; (ii) several years' relevant biomedical research experience; and (iii) a good publication record in international refereed journals with high impact factors. The appointee will (a) participate in research on neuroscience, developmental and stem cell biology, or cancer and cell biology; (b) develop his/her own niche within the aforementioned fields; and (c) teach anatomy courses. The appointee will solicit external research funding. Appointment will normally be made on contract basis for up to three years initially, leading to longer-term appointment or substantiation later subject to budget and mutual agreement.

Salary and Fringe Benefits

Salary will be highly competitive, commensurate with qualifications and experience. The University offers a comprehensive fringe benefit package, including medical care, plus a contract-end gratuity for appointments of two years or longer, and housing benefits for eligible appointees.

Further information about the University and the general terms of service for appointments is available at *http://www.cuhk.edu.hk/personnel*. The terms mentioned herein are for reference only and are subject to revision by the University.

Application Procedure

For post (1): Please send a cover letter, full resume, copies of academic credentials, a publication list and/or abstracts of selected published papers, a research statement and a teaching statement (in pdf format) together with names, addresses and fax numbers/e-mail addresses of at least three referees to whom applicants' consent has been given for their providing references (unless otherwise specified), to *bio_recruit@cuhk.edu.hk*.

For post (2): Please send full resume, copies of academic credentials, a publication list and/or abstracts of selected published papers, together with names, addresses and fax numbers/e-mail addresses of three referees to whom the applicants' consent has been given for their providing references (unless otherwise specified), to the Personnel Office, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong (Fax: (852) 2603 6852) by the closing date.

The Personal Information Collection Statement will be provided upon request. Please quote the reference number and mark "Application - Confidential" on cover.

University of Nebraska Lincoln

IANR

Veterinary and Medical Parasitologist/Entomologist

Departments of Entomology and Veterinary and Biomedical Sciences

Institute of Agriculture and Natural Resources (IANR) University of Nebraska-Lincoln (UNL)

The University of Nebraska-Lincoln is seeking applications to fill a 12-month, tenure-leading, Assistant Professor position for a Veterinary and Medical Parasitologist/ Entomologist in the Departments of Entomology (70% research) and Veterinary and Biomedical Sciences (30% teaching). This position is part of an innovative Professional Program in Veterinary Medicine between UNL and the Iowa State University College of Veterinary Medicine (ISU CVM). For more information and a complete job description, please visit the respective departments' websites: **http://entomology.unl.edu** and **http://vbms.unl.edu**. This position requires a Ph.D. and experience in medical/veterinary entomology, parasitology or related field.

To apply, go to **http://employment.unl.edu**. Search for requisition **#070866**. Complete the faculty academic administrative information form. Attach a letter of application, curriculum vitae, and a list of references. Review of applications will begin **January 4, 2008**, and continue until the position is filled or the search is closed.

The University of Nebraska is committed to a pluralistic campus community through Affirmative Action and Equal Opportunity and is responsive to the needs of dual career couples. We assure accommodation under the Americans with Disabilities Act; contact Marilyn Weidner at 402-472-8679 for assistance.

UtahState UNIVERSITY

Assistant Professor Physiological Ecologist

Assistant Professor [tenure-track; 9-month appointment; Research and Teaching] in the Department of Biology, Utah State University (**http://www.biology.usu.edu**). We seek an animal physiological ecologist whose research addresses whole organism responses to anthropogenic and natural stressors in the environment, with an emphasis on the ecological and evolutionary implications of these responses. Preference will be given to applicants whose research complements established programs in community ecology, physiology, fisheries ecology, macroecology, evolutionary biology, functional genomics, and conservation ecology within the Biology Department (www.biology.usu.edu) and the Ecology Center (www.usu.edu/ecology/). This is a 9-month, tenure track, position with approximately equal emphasis on teaching (undergraduate and graduate) and research. A Ph.D. is required, and evidence of proficiency in both teaching and research will be used as selection criteria. The successful applicant will be expected to establish and maintain an externally funded research program. Teaching responsibilities may include a course in Comparative Animal Physiology and a graduate course in Physiological Ecology. Applicants must apply using the online system at: **http://www.usu.edu/hr/**. Applicants are required to submit: a letter of application stating qualifications and fit to this position, statements of research and teaching goals, curriculum vitae, and names and contact information of three references. Applications will only be accepted through the online system but for further information and inquiries, please contact **Dr. Keith A. Mott, Search Committee Chair, kmott@biology.usu.edu**. Review of applications will begin **14 December 2007** and continue until the position is filled. Utah State University (USU) is a Carnegie-I research institution of over 20,000 students, nestled in a semi-rural mountain valley 80 miles north of Salt Lake City. Housing costs are at or below national averages, and the community provides a supportive environment for families and balanced personal/professional life. USU offers competitive salaries and outstanding medical, retirement, and professional benefits (see **http://www.usu.edu/hr/** for details).

USU is an Affirmative Action/Equal Opportunity Employer, with professional spousal accommodation packages available for dual-career applicants. The University was recently chosen as a National Science Foundation ADVANCE Gender Equity Program recipient and is dedicated to recruiting stellar candidates from a diverse pool including women and minorities.

Faculty Scientists - Pediatrics

The Department of Pediatrics at The University of Texas Medical School at Houston seeks faculty scientists with research interests in the following research areas: complex birth defects, lung development, cardiovascular and neurodevelopmental biology. These research interests will complement those already established in the Department of Pediatrics and allow for collaborations. The University of Texas Medical School at Houston is situated in the Texas Medical Center, the largest medical center in the world composed of 35 medical and research facilities including The University of Texas M.D. Anderson Cancer Center, The University of Texas School of Public Health, and Harris County Hospital District. Facilities within the University include state-of-the-art genomics, proteomics, histopathology and mouse cores and vivarium. Positions are available at the assistant, associate professor and professor levels commensurate with previous experience. These positions are highly competitive with regard to salary, start-up funds and laboratory space. Applicants should have one of the following degrees: PhD, MD or MD/PhD. Successful candidates will be expected to develop and sustain research programs with extramural funding and play an integral role in new program initiatives.

Applicants should send a statement of research interests, curriculum vitae and names and addresses of three references to: **Dr. Jacqueline T. Hecht, PhD, The University of Texas Medical School at Houston, Department of Pediatrics, Rm. 3.316, Houston, TX 77030, Email: Jacqueline.T.Hecht@uth.tmc.edu**

The University of Texas Health Science Center at Houston is an EO/AA employer. M/F/D/V. This is a security sensitive position and thereby subject to Texas Education Code §51.215. A background check will be required for the final candidate.

THE UNIVERSITY of TEXAS
MEDICAL SCHOOL AT HOUSTON
A part of The University of Texas Health Science Center at Houston

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The University of North Carolina at Charlotte
Department of Physics and Optical Science

EXPERIMENTAL BIOPHYSICS TENURE TRACK POSITION

The Department of Physics and Optical Science at the University of North Carolina at Charlotte invites applications for a tenure track Assistant Professor position in experimental Biophysics to start in Fall 2008. Required qualifications include a PhD in Physics or a related field; a strong research record; commitment to teaching physics at the undergraduate and graduate levels to a diverse student population. UNC Charlotte is one of the most rapidly growing universities in the nation, with strong research programs in Nanotechnology, Bioinformatics, Biomedical Engineering Systems, Optoelectronics and Optical Communications, and Precision Metrology. The Physics and Optical Science Department offers PhD and MS programs in Optics, MS programs in Medical Physics and Applied Physics, and is in the process of developing a PhD program in Applied Physics with an emphasis in Biophysics.

Applications must be submitted electronically at **https://jobs.uncc.edu** and must include a CV, contact information of at least three references, and statement of research and teaching interests and goals. Inquiries can be made to the Search Committee Chair, **Prof. Nathaniel M. Fried**, at **nmfried@uncc.edu**. Screening will begin on January 14, 2008 and will continue until the position is filled. For additional information, please visit our website at **www.physics.uncc.edu**.

The University of North Carolina at Charlotte is an EOE/AA Employer and an ADVANCE Institution. Minority and women candidates are encouraged to apply. We are particularly interested in colleagues with diverse backgrounds and experiences who can support the department's strong commitment to developing a deep understanding of and respect for diversity among students and colleagues.

香 港 大 學
THE UNIVERSITY OF HONG KONG

Centenary Recruitment Plan

Founded in 1911, The University of Hong Kong is committed to the highest international standards of excellence in teaching and research, and has been at the international forefront of academic scholarship for many years. Of a number of recent indicators of the University's performance, one is its ranking at 33 among the top 200 universities in the world by the UK's *Times Higher Education Supplement*. The University has a comprehensive range of study programmes and research disciplines, with 20,000 undergraduate and postgraduate students from 50 countries, and a complement of 1,200 academic members of staff, many of whom are internationally renowned.

As the University approaches its 100th anniversary, a major human resource expansion plan has been launched to provide 200 new academic positions. The purpose of this Centenary Recruitment Plan is to enhance our research competitiveness and to facilitate the introduction and delivery of a new four-year undergraduate curriculum from 2012.

Building on Hong Kong's international status and its mission to serve China, the University offers an intellectually-stimulating and culturally-rich academic environment, with attractive remuneration packages.

Tenure-Track Professor/Associate Professor/ Assistant Professor in the Department of Chemistry (Ref.: RF-2007/2008-280)

Applications are invited for tenure-track appointment as Professor/Associate Professor/Assistant Professor (2 posts) in the Department of Chemistry, from August 1, 2008 or as soon as possible thereafter. The posts will initially be made on a three-year fixed-term with the possibility of renewal upon mutual agreement. Appointments with tenure will be considered during the second three-year contract.

Applicants should possess a Ph.D. degree with a strong background and publication record in any discipline of chemistry. Those with research interests that can contribute to the broadly defined areas of Organic Chemistry, Chemical Biology, Materials Science and Theoretical/ Computational Chemistry are especially encouraged to apply. The appointees are expected to develop a vigorous and independent research program and excel in both undergraduate and postgraduate teaching. A suitable start-up fund for research will be provided. Information about the Department can be obtained at http://chem.hku.hk/.

Annual salaries will be in the following ranges (subject to review from time to time at the entire discretion of the University):

Professor	: HK$1.2M
Associate Professor	: HK$622,740 - 963,060
Assistant Professor	: HK$474,600 - 733,440

(approximately US$1=HK$7.8)

At current rates, salaries tax does not exceed 16% of gross income. The appointments will attract a contract-end gratuity and University contribution to a retirement benefits scheme, totalling up to 15% of basic salary, as well as leave, and medical/dental benefits. Housing benefits will be provided as applicable.

Further particulars and application forms (272/302 amended) can be obtained at https://www.hku.hk/apptunit/; or from the Appointments Unit (Senior), Human Resource Section, Registry, The University of Hong Kong, Hong Kong (**fax** (852) 2540 6735 or 2559 2058; **e-mail**: senrappt@hkucc.hku.hk). **Closes December 31, 2007**. Candidates who are not contacted within 4 months of the closing date may consider their applications unsuccessful.

The University is an equal opportunity employer and is committed to a No-Smoking Policy

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LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

FACULTY OF PHYSICS

The **Faculty of Physics** at the Ludwig-Maximilian-University Munich (LMU) invites applications for a

Junior professor position (W1) for Experimental Physics – Nano-Science

The position is originally granted for three years. In case of a positive evaluation it can be extended for further three years.

The desired research field is spectroscopy of solid-based nanoscale systems. Possible topics include spectroscopy of nano-wires and -tubes, quantum dots and wires, hybrid nanosystems, molecular devices and combinations thereof, possibly in combination with scanning probe studies. The research activities of the applicant should complement research of the nanophysics group at Ludwig-Maximilian-University (www.nano.physik-uni-muenchen.de). Access to a range of spectroscopic tools, local probe techniques as well as to clean room facilities is available. Experience in nanoscale fabrication is desirable. The participation in the research program of the Nanosystems Initiative Munich (www.nanoinitiative-munich.de), a federally funded cluster of excellence, as well as related programs is encouraged.

The successful applicant will be expected to have demonstrated excellence in research through completed PhD studies with outstanding results, to have high pedagogical skills, to actively participate in the teaching program of the Department of Physics, and to contribute to new research and teaching initiatives of the department.

The advancement of woman in science is an integral part of the university's policy. Women, therefore, are especially encouraged to apply.

Persons with disabilities will be given preference over other applicants with comparable qualifications.

Applications (including CV with photo, list of publications, description of research accomplishments and interests) are to be sent before **December 31, 2007** to: **Dekan der Fakultät für Physik, Ludwig-Maximilians-Universität, Schellingstraße 4, 80799 München, Germany**.

FLORIDA STATE UNIVERSITY

Open level faculty position in Integrative NanoScience

Florida State University seeks to hire the first of six new faculty in Integrative NanoScience. Current focus of Integrative NanoScience at FSU is nanotechnology that blends "hard" (metals and semiconductors) and "soft"(organic and biological) materials: the science, engineering and art of tailoring and harnessing biomolecular function in nano-fabricated settings. Research is on fundamental nanoscale phenomena and processes that will be required for successful integration of hard and soft materials, and for putting such hybrid materials to practical use. New faculty will complement and extend a highly interactive group of interdisciplinary scientists from materials science, molecular and cell biology, chemical and biomedical engineering, chemistry and biochemistry, and physics. Five additional tenure-track positions at all ranks will be filled over the next few years. Emphasis in hiring will be on faculty exploring: tailored design of biointerfaces; biocompatible nanofabrication; bio-recognition solid-state devices; or molecular transport in micro- and nano-scale devices. See http://www.insi.fsu.edu for further detail. This announcement is part of the Pathways of Excellence Program, an initiative of accelerated growth in focused areas of research aimed at propelling FSU into the top rank of public universities.

Applicants should furnish a curriculum vitae, statements of research and teaching interests, representative publications, and the names and contact information for three references. Applications and inquiries should be addressed to:

Prof. Stephan von Molnár, Chair
Integrative NanoScience Institute Faculty Search Committee
MARTECH and Department of Physics
Florida State University
Tallahassee, FL 32306-4351

E-mail: insi@martech.fsu.edu. Electronic applications encouraged.
Review of applications will begin January 2nd, 2008.
Florida State University is an Equal Opportunity/Affirmative Action employer.

UNIVERSITY OF ILLINOIS
COLLEGE OF MEDICINE AT PEORIA

Associate Professors (Tenured)
University of Illinois College of Medicine at Peoria

The Department of Cancer Biology and Pharmacology at the University of Illinois College of Medicine at Peoria seeks qualified candidates for positions of Associate Professor (tenured). Candidates should have a Ph.D. and/or M.D. degree, a strong publication record, and be actively engaged in an established, extramurally funded laboratory program in the area of molecular genetics, cellular/cancer biology or some aspect of pharmacology. Successful applicants should also have teaching/mentoring experience in directing students, postdoctoral fellows, residents and/or junior faculty in research. Department provides highly competitive salary, benefits and lucrative start-up package. Additionally, a 20,000 square foot dedicated research building with state-of-the-art laboratories is targeted for completion in 2010. Departmental faculty currently have active awards (NIH, DOD, foundations) totaling over $12 million (direct costs) and spanning research interests in cancer biology, genetics, neurotoxicology, Alzheimer's and age-related diseases, alcohol abuse, spinal cord injury and drug development. Many additional collaborative opportunities in basic and clinical research are available within the College and the University.

Applicants should send curriculum vitae and the names and addresses of three references to: **Jasti S. Rao, PhD, Chair, Search Committee, Department of Cancer Biology and Pharmacology, University of Illinois College of Medicine at Peoria, P.O. Box 1649, Peoria, IL 61656**. For fullest consideration, please respond by **January 7, 2008**.

The University of Illinois is an Affirmative Action/ Equal Opportunity Employer.

KECK SCHOOL OF MEDICINE USC

TENURE-TRACK FACULTY POSITIONS
Department of Molecular Microbiology and Immunology
University of Southern California
Keck School of Medicine
Los Angeles, California

The Department of Molecular Microbiology and Immunology at the University of Southern California, Keck School of Medicine is expanding, with a planned recruitment of more than six tenure-track faculty members over the next three years to build upon existing strengths in microbiology and immunology.

The Department invites applicants for **Assistant and/or Associate Professor** positions in the broad fields of microbiology and immunology. We are especially interested in candidates who address pathogenesis-related research topics. Creative scientists with a record of achievement and commitment to excellence in both research and teaching are encouraged to apply. Successful candidates will receive a generous start-up package and laboratory space within our new state-of-the-art research buildings. USC Keck School of Medicine has strong research programs in Cancer, Genomics, Immunology, and Virology.

Applicants should submit a letter of application, curriculum vitae, a statement of current and future research plans, copies of three representative publications, and three letters of recommendation by email to: **calimlim@usc.edu** in PDF format or by mail to:

Dr. Jae Jung, Chair
Department of Molecular Microbiology and Immunology
University of Southern California, Keck School of Medicine
2011 Zonal Avenue, HMR-401
Los Angeles, CA 90033

USC is an Affirmative Action/Equal Opportunity Employer.

Endowed Chair in Nephrology and Hypertension

Dartmouth Medical School and Dartmouth-Hitchcock Medical Center invite applications for the position of Constantine and Joyce Hampers Endowed Chair and Research Director of Nephrology. A physician-scientist active in patient care, graduate medical education and research will hold a joint appointment in the Department of Medicine, a basic science department and, if appropriate, the VA Medical Center. This individual will be responsible for directing, promoting, and developing research programs in Nephrology in collaboration with innovative and nationally recognized programs in basic, translational and clinical research. Substantial resources, including an NIH funded Training Program, are available to support the development of the Nephrology Research Program.

Dartmouth-Hitchcock Medical Center is a 369-bed tertiary care hospital in New Hampshire, with 500 medical and graduate students, and, along with its affiliate VA Medical Center, is a major teaching hospital for Dartmouth Medical School.

The successful candidate will be board certified in Internal Medicine and Nephrology, have an outstanding record of scholarly achievement and sustained extramural research funding, possess excellent interpersonal and mentoring skills, and possess organizational and administrative ability. Applicants must qualify for a senior academic appointment as Associate Professor or Professor of Medicine at Dartmouth Medical School. The committee will begin reviewing applications on **February 15, 2008**. A curriculum vitae, description of current and future research plans, and names of three references should be submitted electronically to:

Bruce A. Stanton, Ph.D.
Chair, Nephrology Search Committee
Professor of Physiology
Dartmouth Medical School
Hanover, NH 03755
bas@Dartmouth.edu

Dartmouth is an Equal Opportunity/Affirmative Action Employer and strongly encourages applications from women and minority candidates.

BCM

Baylor College of Medicine

FACULTY POSITIONS
HUMAN GENOME SEQUENCING CENTER

The Human Genome Sequencing Center (HGSC) at Baylor College of Medicine is seeking to recruit two Junior Faculty members with an interest in Genomics, Bioinformatics, Genetics and/or Cancer. The faculty positions available are independent, tenure track appointments but are expected to complement the ongoing direction of HGSC programs.

The HGSC was founded in 1996 under the leadership of Dr. Richard Gibbs and is a world leader in genomics. The fundamental interests of the HGSC are in advancing biology and genetics by improved genome technologies. As one of the three large-scale sequencing centers funded by the National Institutes of Health, the HGSC provides a unique opportunity to work on the cutting edge of genomic science in a state of the art institution.

Qualified candidates should submit curriculum vitae and three letters of reference via mail, e-mail or fax to:

BCM Human Genome Sequencing Center
Attn: Roxanne Reyna
One Baylor Plaza – BCM 226
Houston, Texas 77030
Email: rbeltran@bcm.tmc.edu
Fax: 713-798-5741

Curriculum vitae and reference letters should be received by **December 31, 2007**.

Our positions are processed through the Baylor College of Medicine Human Resources Department. You will also need to apply for this position by visiting the BCM employment website (position **#160502**): **https://www.medschooljobs.org**.

Baylor College of Medicine is an Equal Opportunity, Affirmative Action, and Equal Access Employer.

CSB Cell & Systems Biology

Systems Biology: Neurobiology
University of Toronto

The new Department of Cell and Systems Biology at the University of Toronto invites applications for a tenure track faculty position to be appointed at the Assistant Professor level in the field of Systems Biology - Neurobiology to begin July 1, 2008.

We particularly encourage candidates to apply who have demonstrated excellence in addressing fundamental questions in neurobiology using high-throughput approaches or gene/protein network analyses with bioinformatics, genomic, proteomic, electrophysiological, or imaging tools. Our vision is to advance Systems-wide analyses in Neurobiology which complement existing strengths in the department (**www.csb.utoronto.ca**). Candidates should have at least two years of research experience beyond their doctoral degree. In addition to pursuing a vigorous, internationally recognized research program, the successful candidate will contribute to undergraduate and graduate teaching in the molecular life sciences. She or he would also be expected to network with researchers across campus to take advantage of the extensive resources in Systems Biology at the University of Toronto and its affiliated institutions. A generous start-up package will be provided. Salary commensurate with qualification and experience.

Applicants should arrange to have at least three letters of recommendation sent directly to the address below. In addition, applicants should forward their curriculum vitae, copies of significant publications, and statements of research and teaching interests to the: **Chair, Systems Biology Search Committee, Department of Cell and Systems Biology, University of Toronto, 25 Harbord Street, Toronto, Ontario M5S 3G5, Canada** by **January 31, 2008**. Inquiries should be directed to **Les Buck** at **buckl@zoo.utoronto.ca**. The University of Toronto offers the opportunity to teach, conduct research and live in one of the most diverse cities in the world, and is responsive to the needs of dual career couples.

The University of Toronto is strongly committed to diversity within its community and especially welcomes applications from visible minority group members, women, Aboriginal persons, persons with disabilities, members of sexual minority groups, and others who may contribute to the further diversification of ideas. All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority.

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

FACULTY OF PHYSICS

To strengthen and widen the scope of the nanosciences at the Ludwig-Maximilians-Universität München, the **Faculty of Physics** and the **LMU*Innovativ* focus area "Functional Nanosystems"** invite applications for a

Full Professorship (W3) for Theoretical Physics

in the area of Theoretical Nanophysics.

Possible fields of research include all questions relevant for understanding, designing and manipulating functional nanosystems. Preferred focus areas are electronic, magnetic, photonic, mechanical or biomimetical nanosystems.

Participation in the interdisciplinary activities of the cluster of excellence "Nanosystems Initiative Munich" (NIM), the "Center for NanoScience" (CeNS), the "Arnold Sommerfeld Center for Theoretical Physics" (ASC), as well as in local collaborative research programs is possible. For additional information on ongoing activities see www.cens.de.

Candidates are expected to conduct an independent research program that complements existing research efforts and to participate in the teaching program.

Prerequisites for employment are a university grade, pedagogical suitability, Ph. D. or doctoral degree and additional scientific qualification which can be proved by a habilitation or equivalent achievements.

In general the age of 52 should not be exceeded at the time of appointment, though exceptions are possible.

The advancement of women in science is an integral part of the university's policy. Women, therefore, are especially encouraged to apply.

Persons with disabilities will be given preference over other applicants with comparable qualifications.

Applications including a curriculum vitae, academic records, a list of publications and invited lectures, as well as a two-page summary of planned research activities should reach the **Dekanat der Fakultät für Physik der Ludwig-Maximilians-Universität München, Schellingstr. 4, 80799 München, Germany,** not later than **December 31, 2007**.

POSITIONS OPEN

CHIEF of GENETICS
University of Arkansas for Medical Sciences
College of Medicine

The College of Medicine (COM) of the University of Arkansas for Medical Sciences (UAMS) invites applications and nominations for the position of Chief of a new free-standing Division of Genetics with the expectation that this Division will grow to attain departmental status in the COM within five years. Responsibilities of the Chief are educational, including oversight of the genetics course for second-year medical students, as well as further development of ambulatory and consulting programs in adult clinical genetics and personalized medicine in addition to evolution of interdisciplinary research programs in genetics.

Candidates must possess a doctoral degree in medicine or human genetics with relevant Board certification and qualifications meriting appointment at the rank of **PROFESSOR.** The ideal applicant will demonstrate excellence in teaching; experience in faculty recruitment and development; and in building a collaborative research program. The position is available in spring 2008.

The COM is one of six colleges at UAMS and is the only medical school in the state. With nearly 1,000 faculty members, the COM provides education and training for over 600 medical students and 593 residents. COM faculty based at UAMS and its affiliate, Arkansas Children's Hospital, have $50 million in NIH funding. In fiscal year 2007, UAMS had nearly 16,000 adult discharges and over 400,000 clinic visits. The UAMS campus is currently expanding with a new 500,000 square-foot hospital, a new education building, a twelve-floor expansion of the Winthrop P. Rockefeller Cancer Institute, and a new Psychiatric Research Institute. For more information, see the UAMS **website: http://www.uams.edu.**

Applications with curriculum vitae, nominations, and requests for information should be directed to:

Aubrey J. Hough, M.D.
Distinguished Professor of Pathology
Associate Dean for Translational Research and Special Programs
College of Medicine
University of Arkansas for Medical Sciences
4301 W. Markham Street, Slot 517
Little Rock, AR 72205
Telephone: 501-686-5369

UAMS is an Equal Opportunity Employer, promoting workplace diversity.

In conjunction with the Center for Marine Science at the University of North Carolina, Wilmington (UNCW), the Laboratory of **Dr. James E. Gibson** at the Brody School of Medicine at East Carolina University is offering an exceptional **RESEARCH FELLOWSHIP** in marine pharmacology/toxicology/biotechnology. Candidates must have a Ph.D. in a marine or a pharmacology/biotechnology-related area and are expected to conduct research in Dr. Gibson's laboratories while pursuing a professional M.B.A. degree in the Cameron School of Business at UNCW. The goal of this 24-month program is to produce individuals with a solid science background as well as the business skills needed to prosper in a modern competitive business environment. Students in the M.B.A. portion of the Program will master the core functions of business, develop analytical and quantitative business skills, and study current and future business issues through real world experiences with regional companies involved in marine biotechnology. Excellent verbal and written skills are required. The research portion of the Program generally involves working in marine pharmaceuticals and nutraceuticals from cultured organisms, bioengineered natural products, novel enzymes, and biosynthetic pathways. Candidates should clearly identify their interests in their cover letter. Candidates with interests in other biotechnology related area will be considered based on the strength of their applications. You can visit **website: http://www.ecu.edu/cs-admin/hr/Employment.cfm** to apply.

POSITIONS OPEN

ASSISTANT PROFESSOR
Department of Cell Biology and Anatomy

The Department of Cell Biology and Anatomy of the Chicago Medical School, Rosalind Franklin University of Medicine and Science, invites applications for a tenure-track Assistant Professor position. We seek candidates to join an ongoing expansion of Department and Medical School research faculty. Areas of research in the Department include the neurobiology of learning and memory, synaptic plasticity in the auditory system, the molecular cell biology of muscle development, cellular trafficking, and extracellular matrix, RNA biology and the role of splicing defects in human disease, and the cellular dynamics of gene expression. Institutional strengths include neuroscience, structural biology, and membrane transport physiology. The successful applicant is expected to establish a strong, extramurally funded research program, and to contribute to medical and graduate teaching. Available core resources include confocal, live-cell and electron microscopy facilities, and structural biology and proteomics centers. Information on the Department may be found at **website: http://www.rosalindfranklin.edu/cms/anatomy.**

Applications containing curriculum vitae, statement of research plans, and the names of three references should be e-mailed to **e-mail: vilmary.friederichs@rosalindfranklin.edu.** Review of applications will begin immediately and will continue until the position is filled. *Rosalind Franklin University of Medicine and Science is an Equal Opportunity/Affirmative Action Employer.*

RESEARCH ASSOCIATE and POSTDOCTORAL POSITIONS for molecular and proteomic studies of nuclear receptors and translation regulators for adipocytes and neurons.

We focus on regulatory mechanisms in stem cell proliferation, adipocyte differentiation, and neuronal protein expression, triggered by nutrients, hormones, and neuronal activity. We investigate factors regulating chromatin remodeling, RNA transport, and translation. Various molecular, cellular, and proteomic approaches are used. For our recent publications, see *Nature Structural and Molecular Biology* **14:**68-75, 2007; *Nature Chemical Biology* **3:**161-5, 2007; *Proc. Natl. Acad. Sci.* **104:** 13810, 2007; *EMBO J.* **26:** 1522-31, 2007; and *Mol. Cell* **19:** 643-53, 2005. A Ph.D. in biological sciences or biophysics is required. Interested applicants should send curriculum vitae via e-mail to **Dr. Li-Na Wei, Department of Pharmacology, University of Minnesota Medical School** at **e-mail: weixx009@umn.edu.** For research details see **website: http://www.pharmacology.med.umn.edu/staffwei.html.** *The University of Minnesota is an Equal Opportunity Educator/Employer.*

NATIONAL UNIVERSITY of SINGAPORE
Department of Chemical and Biomolecular Engineering

The Department of Chemical and Biomolecular Engineering at National University of Singapore invites applications for **TENURE-TRACK FACULTY POSITIONS** at all levels. The Department is one of the largest internationally with excellent in-house infrastructure for experimental and computational research. A Ph.D. in chemical engineering or related areas and a strong research record with excellent publications are required. Please refer to **website: http://www.chbe.nus.edu.sg/** for more information on the areas of interest and for application details. Applicants should send full curriculum vitae (including key publications), a detailed research plan, a statement of teaching interest, and a list of names of at least three references to: **Professor Raj Rajagopalan, Head of Department (attention: Ms. Nancy Chia, e-mail: nancychia@nus.edu.sg).**

POSITIONS OPEN

Dean
College of Natural Resources
University of California, Berkeley

The University of California, Berkeley invites nominations and applications for the position of Dean of the College of Natural Resources. The appointee is expected to join the faculty beginning July 1, 2008, or thereafter. Applications should include a curriculum vitae, bibliography, a brief description of research and other interests, and management accomplishments and objectives. Applications or nominations must be received by **December 1, 2007**.

Please send these materials, along with the names and addresses of three references, to:

Chair, College of Natural Resources
Dean Search Committee
Office of the Vice Provost Academic Affairs
University of California, Berkeley
200 California Hall
Berkeley, CA 94720-1500

Electronic submissions are encouraged and should be sent to:
cnr_dean_search@berkeley.edu

For more information, please visit:
http://vpaafw.chance.berkeley.edu/deansearches.html

The University of California is an Affirmative Action/Equal Opportunity Employer.

FACULTY POSITIONS IN PROTEOMICS

Boston Biomedical Research Institute

The Boston Biomedical Research Institute seeks outstanding investigators in proteomics research to join an exciting new initiative in Integrative Protein Biology. This research program builds on BBRI's existing strength in protein biochemistry and molecular biophysics, incorporating proteomics and systems biology approaches to address challenging biomedical research problems in increasingly comprehensive and innovative ways. Talented scientists who employ proteomics approaches, including but not limited to quantitative mass spectrometry-based techniques, to investigate important problems in biology that have relevance to human disease are encouraged to apply. Candidates must have a Ph.D., M.D., M.D./Ph.D., or equivalent degrees and several years of post-doctoral research experience. Faculty positions are available at ranks equivalent to Assistant, Associate, or Full Professor, commensurate with independent research experience.

BBRI is a dynamic independent research institute located within minutes of all of the Boston area's major universities and medical centers. In addition to the Integrative Protein Biology program, BBRI has recently launched and expanded three disease-focused research programs in Cancer Biology, Cardiovascular Biology, and Regenerative Biology. These new research programs provide unique opportunities for resources and collaboration for faculty in the Integrative Protein Biology program. BBRI is composed of a diverse and highly collaborative group of structural biologists, biochemists, molecular biologists, cell biologists, and mathematical biologists, who utilize state-of-the-art technologies and innovative approaches to further our understanding of molecular mechanisms relevant to biomedicine.

Applicants should send their *curriculum vitae* and a statement of research interests by mail or electronically to the following address. Applicants should also have three letters of reference forwarded to the same address to complete their application.

IPB Faculty Search Committee
Attn: Mrs. Marilyn Sullivan
Boston Biomedical Research Institute
64 Grove St
Watertown, MA 02472

Email: msullivan@bbri.org
Electronic applications are preferred. Deadline for receipt of applications is **January 4, 2008**.

Boston Biomedical Research Institute is an Equal Opportunity Employer.

THE HONG KONG UNIVERSITY OF SCIENCE AND TECHNOLOGY
DEPARTMENT OF BIOCHEMISTRY
Faculty Position

The Department of Biochemistry conducts cutting-edge research in the areas of cellular regulation and signaling, biotechnology and medicinal biochemistry, and macromolecular structure and function (**www.ust.hk/bich/**). Applications for tenure-track faculty position at the Assistant/Associate Professor level are now invited. Applicants should have a PhD degree, relevant postdoctoral experience and a proven record of research excellence.

The Department is interested in appointments to strengthen existing research programs. Preferences will be given to scientists in the areas of (1) molecular and cellular biology with research focus in neuroscience using animal models, and (2) structural biology using X-ray crystallography. Scientists prominent in other areas will also be considered. The appointee is expected to teach both undergraduate and graduate courses. Starting salary will be highly competitive and commensurate with qualifications and experience. Fringe benefits including medical/dental benefits, housing, etc. will be provided where applicable. The gratuity together with the University's total contributions made to the Mandatory Provident Fund Scheme will amount to 15% of the earned aggregate basic salary.

Applications should be sent with CV and names and addresses of three referees to: **Secretary of the Search Committee, Dept of Biochemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong (Email: bcamy@ust.hk) before 16 December 2007 or until the position is filled.**

UCSF

University of California, San Francisco
Tenure Track Faculty Position in Chemical Biology

The Department of Pharmaceutical Chemistry seeks to hire an Assistant Professor interested in problems at the interface of Chemistry and Biology. Of particular interest are those interested in the use of synthetic chemistry to solve problems driven by biology or medicine. The expected appointment is at the level of Assistant Professor (tenure-track). Senior level appointments in exceptional cases will be considered.

Applicants should have a Ph.D., M.D. or advanced degree with research experience and are expected to establish a dynamic research program. Applicants are also expected to actively participate in graduate training in the Chemistry and Chemical Biology Program or other Programs in Quantitative Biology and in professional school teaching. Applicants will be eligible for membership in the Program in Biological Sciences (PIBS) and other graduate training programs.

UCSF seeks candidates whose experience, teaching, research, or community service has prepared them to contribute to our commitment to diversity and excellence.

Please send a *curriculum vitae*, three letters of reference, a summary of current research (up to 3 pages), and a concise outline of future research (up to 3 pages) to the address listed below. Applications will be considered beginning **January 1, 2008**.

Barbara Raymond
Chemical Biology Search Committee
Department of Pharmaceutical Chemistry
University of California San Francisco
600 16th Street, MC 2280
Genentech Hall, Room 518
San Francisco, CA 94143-2517

UCSF is an Affirmative Action/Equal Opportunity Employer. The University undertakes affirmative action to assure equal employment opportunity for underutilized minorities and women, for persons with disabilities, and for covered veterans.

POSITIONS OPEN

FUNCTIONAL ORGANISMAL BIOLOGY of FISHES, REPTILES, or AMPHIBIANS
University of Michigan

The Department of Ecology and Evolutionary Biology solicits applications for a tenure-track **ASSISTANT PROFESSOR,** university-year appointment, in functional organismal biology of ectothermic vertebrates. We seek outstanding individuals whose research involves innovative approaches to studying ectotherm form and function in an evolutionary and/or ecological context, including such fields as evolutionary physiology, physiological ecology, evolutionary or functional morphology, and/or biomechanics. The successful candidate will have complete access to the outstanding collections of the University of Michigan Museum of Zoology and, as appropriate and desired, could have an affiliation with the Museum. Teaching responsibilities may include courses in physiology or anatomy and areas of specialized research interest. For further information, see **websites: http://www.eeb.lsa.umich.edu** and **www.ummz.lsa.umich.edu.** To apply, send curriculum vitae, statements of current and future research plans and of teaching philosophy and experience, evidence of teaching excellence, and copies of publications, as well as arrange to have three reference letters mailed to: **Chair, Functional Biology Search Committee, Department of Ecology and Evolutionary Biology, University of Michigan, 2019S Kraus, 830 N. University, Ann Arbor, MI 48109-1048.** Review of applications will begin on January 5, 2007, and continue until the position is filled. *Women and minorities are encouraged to apply. The University is supportive of the needs of dual-career couples. The University of Michigan is an Equal Opportunity, Affirmative Action Employer.*

Centers for Disease Control and Prevention is seeking candidates under the Senior Biomedical Research Service for the position of **CHIEF,** Laboratory Branch, Division of HIV/AIDS Prevention (DHAP), National Center for HIV/AIDS, Viral Hepatitis, Sexually Transmitted Diseases, and Tuberculosis Prevention (NCHHSTP). This position serves as Manager and Supervisor of four laboratory teams of 85 laboratory and administrative staff in three laboratory facilities in HIV research for DHAP including intervention studies, diagnostic development and incidence surveillance, drug resistance surveillance, international support, and non-HIV retroviral surveillance. Other responsibilities include basic and epidemiologic investigations into known and new human retroviruses using molecular biologic, immunologic and virologic methodologies as they pertain to public health. Please refer to the **website: http://jobsearch.usajobs.opm.gov/getjob.asp?JobID=65068203&AVSDM=2007%2D11%2D09+00%3A00%3A07&Logo=0&q=Chief,+Laboratory+Branch&lid=392&FedEmp=Y&sort=rv&vw=d&brd=3876&ss=0&FedPub=Y&SUBMIT1.x=77&SUBMIT1.y=17.**

MICROBIOLOGIST

The Department of Biology at the University of West Georgia invites applications for a tenure-track position at the rank of **ASSISTANT PROFESSOR** with a starting date of August 2008. A doctoral degree and record of research productivity are required. Postdoctoral training and teaching experience are preferred. Teaching responsibilities may include introductory biology, upper-level courses in microbiology, and advanced courses in the candidate's expertise such as immunology and virology. Please submit a cover letter, current curriculum vitae, teaching philosophy and research interests/plans, copies of academic transcripts and three letters of recommendation to: **Dr. S. Swamy Mruthinti, Professor and Chair of Search Committee, Department of Biology, University of West Georgia, Carrollton, GA 30118.** Review of completed applications will begin on January 7, 2008, and continue until the position is filled. *UWG is an Equal Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

OREGON HEALTH&SCIENCE UNIVERSITY — SHRINERS RESEARCH CENTER

POSTDOCTORAL POSITIONS

Two positions are now available to characterize the function of Hox proteins in development. Qualified applicants should have expertise in one or more of the following areas: whole embryo culture, protein-DNA interaction, transcriptional regulation of eukaryotic gene expression, in situ hybridization, qRTPCR, immunohistochemistry, in vitro gene expression assays, site directed mutagenesis, or chromatin immunoprecipitation.

Curriculum vitae, samples of first author publications, and the names of three references should be mailed to: **H. Scott Stadler, Ph.D., Shriners Hospital for Children, Mail Code SHC-Research, 3101 S.W. Sam Jackson Park Road, Portland, OR 97239** or to **e-mail: hss@shcc.org.** *OHSU and the Shriners Hospitals for Children are Equal Opportunity Employers, Drug-Free, and Smoke-Free Employers.*

FACULTY POSITION in BIOPHYSICS
Department of Physics and the Center for Cell and Genome Science
University of Utah

The Department of Physics and the Center for Cell and Genome Science invite applications for a tenure-track faculty position in biophysics at the **ASSISTANT PROFESSOR** level. More senior applicants seeking a higher-level appointment are also welcome to apply. We seek creative and independent **PHYSICISTS** who are working in any area of cell biology or genome science. We are particularly interested in scientists who are pursuing quantitative, mechanistic approaches to fundamental problems in biology, including the application of novel methods from the physical sciences. Successful applicants will be expected to establish a vigorous, independent research program and contribute to teaching. New faculty will have access to graduate students from programs in biology, molecular biology, biological chemistry, neuroscience, and physics and will be provided with outstanding infrastructural support. Please send curriculum vitae, representative publications, and three letters of reference to: **Andres V. Maricq, Chair, c/o Melissa Brown (e-mail: melissabrown@bioscience.utah.edu), Center for Cell and Genome Science Search Committee, Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112-0840.** Candidates must hold a Ph.D. degree or equivalent, preferably in physics or biophysics. Review of applications will commence December 1, 2007, and will continue until the position is filled. *The University of Utah is an Equal Opportunity/Affirmative Action Employer, and encourages applications from women and minorities and provides reasonable accommodation to the known disabilities of applicants and employees. The University of Utah values candidates who have experience working in settings with students from diverse backgrounds and posses a strong commitment to improving access to higher education for historically underrepresented students.*

POSTDOCTORAL FELLOW - JOHNS HOPKINS UNIVERSITY SCHOOL of MEDICINE

Within the Department of Radiology and Institute for Cell Engineering. Candidate has strong background in experimental neuroscience and stem cell biology for position funded by the Maryland State Stem Cell Fund. Will use human embryonic stem cells for treatment of multiple sclerosis in mouse experimental allergic encephalomyelitis models, with MR and bioluminescent imaging to track cells non-invasively. Expertise with neurosurgery, histology, and stem cell cultures a must. Although desirable, imaging experience not required. Contact: **Dr. Jeff W.M. Bulte, Director of Cellular Imaging, Johns Hopkins University School of Medicine, e-mail: jwmbulte@mri.jhu.edu, website: http://www.hopkins-ice.org/vascular/int/bulte.html.**

POSITIONS OPEN

ASSISTANT/ASSOCIATE PROFESSOR

The Department of Physiology of the Brody School of Medicine at East Carolina University (ECU) is seeking applicants for three tenure-track faculty positions due to retirement and expansion. The Department currently has 11 full-time faculty members. Descriptions of the Department and the research interest of the faculty are available at **website: http://www.ecu.edu/physio.** Applicants must have a Ph.D., or M.D., or equivalent degree and appropriate postdoctoral training. While individuals with expertise in any area of physiology will be considered, preference will be given to candidates conducting interdisciplinary research in the following areas: cardiovascular, pulmonary, neuroscience, metabolism, and exercise physiology. The successful candidates will have a strong publication record and will be expected to develop an extramurally funded research program and to support and mentor graduate students in the Physiology and/or the Bioenergetics Programs **(website: http://www.ecu.edu/hhp).** Successful applicants will have an understanding of organ systems physiology and contribute to team-taught courses for medical, allied health, and graduate students. A competitive startup package, including guaranteed salary support, is available. Interested applicants should submit curriculum vitae, a short description of their research goals, teaching experience, and the names of three references through the online ECU-Human Resources site, **website: https://ecu.peopleadmin.com.** Search for positions using Physiology in the department field. The specific job opening is #75901. Address any questions to: **Robert M. Lust, Ph.D., Professor and Chair, Department of Physiology, e-mail: lustr@ecu.edu, office telephone: 252-744-2762**. A start date of July 2008, is anticipated, and review of applicants will continue until the positions are filled. *ECU is an Equal Opportunity/Affirmative Action University and accommodates individuals with disabilities. Applications are particularly welcomed from women and members of underrepresented minority groups. Proper documentation of identity and employability are required at the time of employment.*

The College of Arts and Sciences, Loyola University Chicago, invites applications for **CHAIRPERSON of the DEPARTMENT of BIOLOGY,** effective August 15, 2008. The Department has research strengths in molecular developmental/evolutionary biology, neurobiology and aquatic ecology, serves nearly 1,400 undergraduate majors, and offers M.S. and M.A. degrees. A highly active research faculty is supported by an extensive infrastructure, including state-of-the-art laboratories and other research facilities. For further information about the Department, visit our **website: http:// www.luc.edu/biology.**

Applicants with expertise in neurobiology are preferred; candidates doing research in the other areas listed above are also encouraged to apply. Candidates should qualify for tenure as a **FULL PROFESSOR** and have a strong externally funded research program, possess demonstrable leadership abilities, outstanding interpersonal skills, and a vision for enhancing the Department's research and educational programs. Candidates should apply online at **website: http://www.careers.luc.edu** with current curriculum vitae, summary of research support; names and addresses of three recommenders; and a statement about research, service, teaching, and administrative interests.

Review of applications will begin December 1, 2007, and continue until the position is filled. Written inquiries about the position can be sent to the: **Biology Chairperson Search Committee, Department of Biology, Loyola University Chicago, 6525 N. Sheridan Road, Chicago, IL 60626.**

Loyola University Chicago, the country's largest Jesuit Catholic university, has nine schools and colleges on three campuses. For further information about the University, consult **website: http:/www.luc.edu.** *Loyola University Chicago is an Equal Opportunity/Affirmative Action Employer with a strong commitment to diversifying its faculty. Applications from women and minority candidates are especially encouraged.*

POSITIONS OPEN

FACULTY POSITIONS
Biochemistry/Molecular Biology, Ecology, and Health Sciences

The Department of Biological Sciences at Michigan Technological University invites applications for two or more positions in the first round of an anticipated series of hires highlighting the integrative future of biology. One position will be in biochemistry/molecular biology; the other position(s) will be in ecology or health sciences complementing current departmental strengths and goals. Appointments are at the **ASSISTANT PROFESSOR** level; however, exceptionally qualified applicants may be appointed at the **ASSOCIATE PROFESSOR** level. For all these hires, we are particularly interested in individuals who conduct research at the interfaces of ecology, biochemistry/molecular biology, and/or human health. Successful applicants will be expected to establish a vigorous, externally funded research program involving graduate and undergraduate students and to have a strong commitment to undergraduate and graduate instruction in their areas of expertise. Additional information is available at **website: http://www.bio.mtu.edu.** Applicants should submit curriculum vitae, statements of research and teaching interests, and three letters of recommendation to: **Chair, Faculty Search Committee, Department of Biological Sciences, Michigan Technological University, Houghton, MI 49931; e-mail: biosearch@mtu.edu.** Review of applications will begin December 15, 2007, and continue until all positions are filled.

At Michigan Technological University, sustainability informs research at a university-wide scale. Candidates who are also interested in research that fits within the theme of sustainability should send a separate application for one of the ten growth positions in that area as described at **website: http://www.mtu.edu/sfhi.** *Michigan Technological University is an Equal Opportunity Educational Institution/Equal Opportunity Employer.*

FACULTY POSITION in METABOLOMICS or STRUCTURAL BIOLOGY

The Department of Biochemistry and Molecular Biology at the Penn State University College of Medicine invites applications for a full-time, tenure-track position. We seek candidates at any level who are using state-of-the-art, nuclear magnetic resonance-based methods to study the effects of human disease on metabolism (metabolomics) and/or the role of protein structure/function in disease. For additional information, please visit this **website: http://www.hmc.psu.edu/biochemistry/.** Interested applicants should submit curriculum vitae and a brief statement of research plans, and arrange to have three letters of reference sent to: **Judith S. Bond, Ph.D., Professor and Chair, Department of Biochemistry and Molecular Biology H171M, P.O. Box 850, Penn State University College of Medicine, Hershey, PA 17033.** *Penn State is committed to Affirmative Action, Equal Opportunity, and the diversity of its workforce.*

University of Wisconsin, Oshkosh seeks tenure-track **ASSISTANT PROFESSOR in BIOLOGY** with specialty in animal cell physiology, beginning September 1, 2008. Ph.D. required; postdoctoral and teaching experience desirable. Responsibilities: share in teaching courses in molecular and cell biology, animal and human physiology, introductory biology; develop a research program in cell physiology; pursue extramural funding; supervise M.S. theses; advise majors. Send letter of application, brief statements of teaching philosophy and research interests, curriculum vitae, reprints, three current letters of recommendation, and transcripts (official or photocopy) to: **Chair, Department of Biology and Microbiology, University of Wisconsin Oshkosh, Oshkosh, WI 54901.** Deadline: January 3, 2008. At least one letter of recommendation should come from the candidate's current institution. For additional information, see the departmental web page at **website: http://www.uwosh.edu/departments/biology/.** Employment will require a criminal background check. *Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

UAB

DEPARTMENT of MEDICINE
Division of Cardiovascular Disease

The Division of Cardiovascular Disease at the University of Alabama at Birmingham is seeking applicants at the **ASSISTANT PROFESSOR** level, in either a tenure or nontenure track, to join a rapidly expanding program in cardiovascular biology research. The successful candidate will be expected to develop an independent and funded research program. Please send curriculum vitae, letters from three references, and a brief statement of your research interest to: **Robert C. Bourge, M.D., Director, Division of Cardiovascular Disease, University of Alabama at Birmingham, 311 Tinsley Harrison Tower, UAB Station, Birmingham, AL 35294.** *UAB is an Affirmative Action/Equal Opportunity Employer.*

FACULTY POSITION in INFECTIOUS DISEASES
Fred Hutchinson Cancer Research Center and the University of Washington

The Fred Hutchinson Cancer Research Center (FHCRC) and the University of Washington (UW) are jointly recruiting a full-time faculty position at the **ASSISTANT or ASSOCIATE MEMBER/PROFESSOR** level for a faculty position in the Program in Infectious Diseases in the Clinical Research Division of the FHCRC and the Division of Allergy and Infectious Disease at the UW, with preference being given to those who study fungal diseases. A Doctorate degree is required. The individual will be expected to develop independent research programs. University of Washington faculty engage in teaching, research, and service. Excellent collaborations are available with scientists in clinical, molecular medicine, and basic sciences at the FHCRC and the UW. Salary depends on experience and excellent benefits. Interested candidates may submit curriculum vitae, a concise statement of their research plan, and three reference letters to:

Lawrence Corey, M.D.
Head, Program in Infectious Diseases
Fred Hutchinson Cancer Research Center
1100 Fairview Avenue N., LE-500
P.O. Box 19024
Seattle, WA 98109

Application review will continue until the position is filled.

The University of Washington and the Fred Hutchinson Cancer Research Center are Affirmative Action, Equal Opportunity Employers, dedicated to the goal of building a culturally diverse and pluralistic faculty and staff committed to teaching and working in a multicultural environment, and strongly encourage applications from women, minorities, individuals with disabilities, and covered veterans.

DIRECTOR of SEA GRANT PROGRAM

Director, New York Sea Grant Institute. The State University of New York (SUNY) and Cornell University invite nominations and applications for Director of the New York Sea Grant Institute (NYSGI). NYSGI's mission is to develop and deliver science that addresses issues of New York's marine and Great Lakes coasts. Visit our **website: http://www.seagrant.sunysb.edu,** to learn more about our Programs. Based at Stony Brook University, the Director provides leadership to ensure that the research, education, and outreach programs of NYSGI continue to be among the nation's best. For a full job description and to apply online visit **website: http://www.stonybrook.edu/jobs.**

Applications will be accepted until the position is filled. *SUNY is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

FACULTY POSITIONS - TRANSLATIONAL OBESITY/METABOLIC SYNDROME/DIABETES RESEARCH
University of Missouri, Columbia
Departments of Nutritional Sciences/Medical Pharmacology and Physiology

The Department of Nutritional Sciences and the Department of Medical Pharmacology and Physiology, University of Missouri, Columbia invite applications for two tenured or tenure-track positions (one expected at the **ASSISTANT PROFESSOR** level and one at open rank; rank commensurate with experience) from investigators whose interests focus on obesity and its complications. The newly expanded Department of Nutritional Sciences now spans three colleges within the University and has a mission focused on an interdisciplinary approach to the obesity epidemic spanning from pipette to patient to population to policy. The University is noted for interdisciplinary research programs. We are seeking outstanding clinician scientists or nonclinician scientists with translational research programs. Position qualifications include an M.D./Ph.D., M.D., or a Ph.D. with postdoctoral experience and a translational research focus. Successful applicants will have or develop an outstanding research program and contribute to School of Medicine teaching activities. Involvement in campus-wide research initiatives relative to obesity, cardiovascular biology, exercise, metabolism, cell signaling, and nutrition is desirable. Located midway between St. Louis and Kansas City, Columbia is a vibrant university town that is consistently ranked among the top small cities to live in America. Please send curriculum vitae, a narrative of research and educational interests, and the names and contact information of three references to:

Chair, Nutrition/Medical Pharmacology and Physiology Search Committee
Department of Nutritional Sciences
School of Medicine/HES/CAFNR
217 Gwynn Hall
University of Missouri-Columbia
Columbia, MO 65211

Or by electronic submission (strongly preferred) to:
e-mail: umchesnsjobs@missouri.edu

Active review of applications will begin January 15, 2007, and the search will continue until the positions are filled. Visit the University of Missouri-Columbia's **website: http://www.missouri.edu/.** *The University of Missouri-Columbia is an Equal Opportunity Employer, Affirmative Action Employer, and complies with the guidelines set forth in the Americans with Disabilities Act of 1990. Please direct ADA accommodation requests to our coordinator at* ***telephone: 573-884-7278 (V/TTY).***

TWO TENURE-TRACK ASSISTANT PROFESSORS

The Department of Ecology and Evolutionary Biology, Tulane University, invites applications for two tenure-track positions to be filled at the Assistant Professor level: one in global change biology, wetland ecology, or tropical biology; and one in computational biology preferably involving theoretical ecology, ecology and evolution modeling, or landscape ecology. See **website: http://www.tulane.edu/~ebio/news/new-positions.php** for more details about these positions and the Department. Send a letter of application indicating the position, curriculum vitae, statements of research and teaching interests, selected publications, and names and addresses of three references to: **Faculty Searches, Department of Ecology and Evolutionary Biology, 400 Lindy Boggs Center, Tulane University, New Orleans, LA 70118-5698.** Review of applications will begin soon after January 1, 2008, and the searches will remain open until the positions are filled. These positions are subject to a final University determination on funding. *Tulane University is an Affirmative Action/Equal Employment Opportunity Employer.*

POSITIONS OPEN

California State University, Fresno, Department of Biology, is searching for a tenure-track **ASSISTANT PROFESOR in SYSTEMS/COMPUTATIONAL BIOLOGY** to begin August 2008. The successful candidate is expected to develop a research program in a current area of systems biology, including quantitative manipulation of genomic, proteomic, and/or metabolomic datasets from complex physiological networks to investigate basic questions at any level of biological organization from molecular to tissue level to whole organism. Additionally, the successful candidate will be expected to teach an upper-division majors/service course on a rotational basis, and an undergraduate and a graduate course in their areas of expertise (e.g. bioinformatics). An earned Doctorate (Ph.D.) in biology or related field is required. Strong preference will be given to candidates with postdoctoral experience. Send completed application, including form available at **website: http://www.csufresno.edu/aps/vacancy/sc1.pdf,** a cover letter, curriculum vitae, statements of teaching and research philosophy, and three original current letters of reference (dated within the last 12 months) to: **Dr. Frederick Zechman, Committee Chair, Department of Biology, California State University, Fresno, 2555 E. San Ramon Avenue, M/S SB73, Fresno, CA 93740-8034,** or to **e-mail: zechman@csufresno.edu, telephone: 559-278-4095, fax: 559-278-3963.** For full consideration, all materials must be received by February 8, 2008. For more information, visit our **website: http://www.csufresno.edu/biology.** *California State University, Fresno is an Equal Opportunity Employer.*

IMMUNOLOGISTS
University of Georgia

The Department of Infectious Diseases in the College of Veterinary Medicine invites applications for two tenure-track **PROFESSOR** positions in the areas of immunology or host cellular response to infectious diseases. Applicants must have a Ph.D., D.V.M., M.D., or equivalent advanced degree, a minimum of two years of postdoctoral experience, and a strong record of research productivity. The successful candidates will be expected to establish nationally recognized and competitive externally funded research programs and contribute to teaching in the undergraduate, graduate, or professional curricula. Potential applicants can find details at **website: http://www.vet.uga.edu/id/immunologysearch.htm.** Please submit a statement of research plans and teaching philosophy, curriculum vitae, and the names and contact information for at least three references to: **Immunology Search Committee, Department of Infectious Diseases, University of Georgia, Athens, GA 30602.** Applications received before January 15, 2008, are assured full consideration. *The University of Georgia is an Equal Opportunity/Affirmative Action Employer.*

RESTORATION ECOLOGIST
Archbold Biological Station

Archbold Biological Station **(website: http://www.archbold-station.org),** Florida, seeks **ASSISTANT/ASSOCIATE RESEARCH BIOLOGIST** with research interests in restoration ecology. Successful candidate to develop independent research program focused on 3,648-acre Archbold Reserve with wetland, grassland, scrub, and hydrological restoration potential. We are particularly interested in responses of organisms, populations, and communities to restoration. Permanent funding covers salary, full benefits, laboratory, and field facilities. Appointee expected to obtain additional outside funding for program growth. Ph.D. and strong research record required. Full description at **website: http://www.archbold-station.org/abs/staff/jobs.htm.** Send letter, curriculum vitae, statement of how research accomplishments relate to this position, and up to five relevant reprints, along with full contact information for four references to **e-mail: hswain@archbold-station.org** by January 4, 2008. *ABS is an Equal Opportunity Employer.*

POSITIONS OPEN

UAF
UNIVERSITY OF ALASKA
FAIRBANKS

The School of Fisheries and Ocean Sciences (SFOS) at the University of Alaska Fairbanks (UAF) invites applications for two **MARINE BIOLOGIST** vacancies specializing in early life ecology and mammalogy/population genetics. Both positions are **ASSISTANT/ASSOCIATE PROFESSOR,** tenure-track faculty positions within the SFOS Institute of Marine Science located in Fairbanks, Alaska. To learn more about the School please visit **website: http://www.sfos.uaf.edu.** Complete position information can be found at **website: https://www.uakjobs.com,** reference postings 0054202 and 0054183.

ASSISTANT PROFESSOR MICROBIOLOGIEST
Department of Biology
University of Virginia, Charlottesville, Virginia

The Department of Biology at the University of Virginia has an opening for a tenure-track Assistant Professor beginning August 25, 2008. Applications are invited from outstanding individuals studying fundamental aspects of microbiology at the molecular, cellular, organismal, or systems level. Our Department **(website: http://www.virginia.edu/biology/)** spans a broad range of interests including cell and developmental biology, morphogenesis, neurobiology, biological timing, and evolutionary biology. The successful candidate is expected to establish a vigorous, independent, and externally funded research program, interact with one or more existing departmental strengths, and contribute to undergraduate and graduate instruction and training in microbiology. A generous startup package and excellent research facilities are available. Please apply through the University of Virginia online application system at **website: http://jobs.virginia.edu/applicants/Central?quickFind=53210.** Please attach your curriculum vitae, a statement of current and future research interests, and a statement of teaching experience and goals. Please have three letters of recommendation also submitted to **e-mail: biosearch@virginia.edu.** Inquiries about the position may be e-mailed to **e-mail: biosearch@virginia.edu.** Review of applications by the committee will begin December 1, 2007. The position will remain open until filled. *Women and members of underrepresented groups are encouraged to apply. The University of Virginia is an Equal Opportunity/Affirmative Action Employer.*

NEWS EDITOR/WRITER, ASSOCIATION for COMPUTING MACHINERY (ACM) MAGAZINES. Leading technology magazine publisher is seeking a full-time News Editor/Writer for its flagship publication the Communications of the ACM. Responsibilities include authoring high quality, original monthly news articles for the global computer science and information technology community, conducting interviews with researchers and practitioners on topics of broad interest to the community, hiring and managing a network of freelance technology writers, and managing the news section of the magazine's website, which will include frequent updating of content, news feeds, and other third party news content appropriate for distribution online. A minimum of five years of experience as a News Editor/Writer for a technical publication is required. Familiarity with the computing and information technology industry is highly desirable. Competitive compensation and benefits package offered commensurate with experience. Only resumes with cover letter and salary requirements will be considered. Please send resumes to **e-mail: hr-dept@acm.org.**

POSITIONS OPEN

ASSISTANT or ASSOCIATE PROFESSOR
Kansas State University
Plant Molecular Biology

The Division of Biology at Kansas State University invites applications for a tenure-track Assistant or Associate Professor position beginning in the 2008/2009 academic year. We seek an individual who will establish an outstanding, extramurally funded research program within the general area of plant molecular biology. Minimum requirements for an appointment at the Assistant Professor rank include a Ph.D. degree and postdoctoral experience. Minimum requirements for an appointment at the Associate Professor rank include a Ph.D. degree and postdoctoral experience, plus an independent, nationally recognized research program, with current extramural funding, and demonstrated excellence in teaching. The position includes a competitive salary and startup package. For more information go to **website: http://www.ksu.edu/biology/bio/news.htm.** Applicants should indicate rank at which they wish to be considered, and should submit (either electronically or by mail) comprehensive curriculum vitae, statement of research and teaching interests, representative publications, and have three letters of reference sent to: **Dr. Ruth Welti, Chair, Plant Molecular Biology Search Committee, Division of Biology, 116 Ackert Hall, Kansas State University, Manhattan, KS 66506-4901. E-mail: kbiology@ksu.edu.** Review of applications will begin December 3, 2007, and continue until the position is filled. *KSU is an Equal Opportunity/Affirmative Action Employer.*

The Department of Biology at California State University, Fresno invites applications for a tenure-track **ASSISTANT PROFESSOR** position in evolutionary developmental biology to begin August 2008. Applicants must have a Ph.D. and postdoctoral research experience. Candidates whose research targets experimental approaches to evolutionary developmental biology, including but not limited to epigenetic processes, developmental constraints, and genetic regulation of ontogeny, are encouraged to apply. The successful candidate will be expected to establish an active, externally funded research program that includes mentoring undergraduate and Master's students. The successful applicant will contribute to the teaching mission of the Department in the areas of developmental biology, evolution, and their area of specialization. To apply, complete the form at **website: http://www.csufresno.edu/aps/vacancy/Sc1.pdf** and mail hardcopies of a cover letter stating your interest in the position, curriculum vitae, research statement including future goals, teaching philosophy, and have three letters of reference sent to: **Dr. Brian Tsukimura, Committee Chair, Department of Biology, Califomia State University Fresno, 2555 E. San Ramon Avenue, M/S SB73, Fresno, CA 93740.** Questions about the position may be addressed to **Dr. Brian Tsukimura** at **e-mail: briant@csufresno.edu** or **telephone: 559-278-4244.** Review of applications will begin January 17, 2008, and continue until the position is filled. For more information, visit our **website: http://www.csufresno.edu/biology.** *Califomia State University, Fresno is an Equal Opportunity Employer.*

Yale University School of Medicine is seeking a **SCIENTIST** to conduct research related to the effects of human aging on innate immunity. Applicants must have expertise in immunohistochemistry, fluorescent and confocal microscopy, flow cytometry, genomic microarray analysis, and molecular immunology. Stipends: $50,613 with medical and disability insurance. Minimum requirements: Doctoral degree with four years of experience.

Send application to: **Albert Shaw, Yale University Medical School, 300 Cedar Street, TAC S169C, CT 06520-8022, e-mail: albert.shaw@yale.edu.**

POSITIONS OPEN

2007-2008 ELECTRICAL ENGINEERING SEARCH

The Electrical Engineering Department at the University of Washington (UW) seeks outstanding individuals for tenure-track positions primarily at the **ASSISTANT PROFESSOR** level. Particular areas of interest include, but are not limited to: (1) design, modeling, and control of structures, devices, and systems for molecular, quantum, and nano systems; (2) advanced component and system-level design of networked radios and sensors for future ubiquitous global networks; associated architectural and protocol stack integration issues; (3) theory and methods of signal processing and learning for applications such as, but not limited to, cellular systems, language, remote sensing, robotics, or vision.

The ideal candidate will be conversant in the languages of multiple disciplines, grounded in the fundamental underlying sciences, and driven by system applications especially in the emerging areas of life sciences and environmental monitoring. He/she will complement our existing strengths, bridge between various departmental groups and research activities, build a world-leading research program, and provide innovation and quality teaching at both the undergraduate and graduate levels. Applicants must have a Ph.D. or Doctorate degree by the date of appointment.

UW has the highest level of federal funding of all public universities, and the second highest among all universities in the nation. The Electrical Engineering Department currently has 37 tenure-track faculty (30 men/seven women). External research funding of the Department in 2006-2007 was over $15 million (See **website: http:// www.ee.washington.edu).** The Department is committed to outstanding research, teaching and service.

Please submit your resume, list of publications, statement of research and teaching interests and goals, and the names and addresses of at least five references on our **website: http://www.ee.washington.edu/facsearch/.** Applications will be accepted until February 1, 2008, or until the positions are filled. For any administrative issues related to the search, please contact **Ann Langford-Fuchs (e-mail: annf@ee.washington.edu).**

The University of Washington is building a culturally diverse faculty and strongly encourages applications from female and minority candidates. The University of Washington is the recipient of a 2006 Alfred P. Sloan Award for Faculty Career Flexibility and a 2001 National Science Foundation ADVANCE Institutional Transformation Award to increase the advancement of women faculty in science, engineering, and math (see ***website: http://www.engr.washington.edu/advance).*** *The University is an Equal Opportunity, Affirmative Action Employer and is responsive to the needs of dual-career couples.*

The Department of the Geophysical Sciences at the University of Chicago seeks applications for one or more tenure-track faculty positions. We have recently made new appointments in a number of fields, including atmospheric chemistry, biogeochemistry, cosmochemistry and meteoritics, and paleobiology. We are interested in promising candidates in all areas of Earth science who study fundamental processes governing the state and evolution of the Earth and planets. Appointments will generally be at the level of **ASSISTANT PROFESSOR,** but we will consider more senior appointments in exceptional cases. Candidates must have completed the Ph.D. prior to appointment. Please send curriculum vitae, statement of research and teaching interests, and contact information for three or more references to: **Michael Foote, Chair, Department of the Geophysical Sciences, The University of Chicago, 5734 South Ellis Avenue, Chicago, IL 60637 U.S.A.** Consideration of applications will begin November 1, 2007. *The University of Chicago is an Affirmative Action/Equal Opportunity Employer.*

RESEARCH ASSISTANT with experience in molecular cloning. Send resume to: **Q. Kong, Case Western Reserve University, Department of Pathology, 2085 Adelbert Road, Cleveland, OH 44106.** Must reference job code FF5007.

ASSISTANT PROFESSOR, MEDICAL and VETERINARY EXTENSION ENTOMOLOGY. Twelve-month, tenure track. The Department of Entomology, Kansas State University, seeks applicants for the position of Assistant Professor of Medical and Veterinary Extension Entomology (70 percent extension, 30 percent research) to develop a nationally recognized extension program dealing with arthropods that affect humans, livestock, and other animals in Kansas and surrounding regions. Incumbent is expected to establish an extramurally funded research program in medical and veterinary entomology focused on management of arthropod pests relevant to clientele associated with extension and outreach responsibilities. Ph.D. degree in medical and/or veterinary entomology or other relevant discipline and evidence of effective communication skills are required. Postdoctoral experience is desirable. A detailed position announcement is available at **website: http://www.entomology.ksu.edu.** Applications must include a statement of extension and research background, interests and philosophy, detailed curriculum vitae, up to five relevant publications, transcripts, and three letters of recommendation. Mail applications to: **Dr. C. Michael Smith, Chair, Search Committee, Department of Entomology, Kansas State University, Manhattan, KS 66506-4004 (voice: 785-532-4700, fax: 785-532-6232, e-mail: cmsmith@ksu.edu).** Electronic applications will not be accepted. Review of applications begins January 1, 2008, and will continue until a suitable candidate is identified. *Kansas State University is an Equal Opportunity/Affirmative Action Employer. Women and minorities are particularly encouraged to apply.*

ASSISTANT PROFESSOR, ARTHROPOD VECTOR BIOLOGY. Twelve-month, tenure track. The Department of Entomology, Kansas State University seeks applicants for the position of **ASSISTANT PROFESSOR of VECTOR BIOLOGY** (90 percent research, 10 percent teaching). Research: maintain extramurally funded research on arthropods that transmit human and animal parasites/pathogens. Preferred areas of research include, but are not limited to, bioinformatics, genomics, and epidemiology. Teaching: training of students, teaching an undergraduate course in medical entomology and a graduate course in area of interest. Ph.D. in entomology or related field with training in vector biology required. Postdoctoral experience preferred. Complete description available at **website: http://www.entomology.ksu.edu/DesktopDefault.aspx?tabid=38.** Screening of applicants will begin January 4, 2008, and continue until a suitable candidate is found. Please send curriculum vitae, statement of research and teaching interests, five relevant reprints, and three letters of recommendation to: **Dr. Srini Kambhampati, Chair, Vector Biology Search Committee, Department of Entomology, Kansas State University, Manhattan, KS 66506.** Electronic applications will not be accepted. *KSU is an Affirmative Action/Equal Opportunity Employer.*

UCLA SCHOOL of PUBLIC HEALTH: DIRECTOR, Center to Combat Emerging Infectious Diseases. Tenure-track faculty member to serve as the Director for the new Center to Combat Emerging Infectious Diseases (CCEID). Seeking background in: epidemiology, virology, microbiology, informatics, or a related field, whose work is relevant to combating emerging infectious diseases. Candidates must have a doctoral or equivalent degree, international prominence in the field of infectious diseases, and demonstrated leadership abilities. A complete job description is available at **website: http://www.ph.ucla.edu/about_hr_jobs.html.** Please send curriculum vitae and letter of interest to: **Ms. Susan Fisher, Coordinator, Faculty Searches, Office of the Dean, UCLA School of Public Health, P.O. Box 951772, Los Angeles, CA 90095-1772.** The Search Committee will begin considering applications November 15, 2007, and will continue until the position is filled. *Affirmative Action/Equal Opportunity Employer. Applications from women and underrepresented minority candidates are especially welcome.*

FACULTY POSITION in BIOMOLECULAR and/or BIOPHYSICAL CHEMISTRY

The Department of Chemistry and Biochemistry at Arizona State University (ASU) invites applications for a tenure-track position at the rank of **ASSISTANT PROFESSOR in BIOPHYSICAL and/or BIOMOLECULAR CHEMISTRY.** Duties include establishing a vigorous, externally funded research program of national/international recognition, teaching chemistry or biochemistry courses at the graduate and undergraduate levels, and participating on assigned governance and service committees in the Department of Chemistry and Biochemistry **(website: http://chemistry.asu.edu/).** A successful candidate must have a doctoral degree in chemistry, biochemistry, or related field. Postdoctoral experience is desired. Preference will be given to individuals studying fundamental problems in biological systems using structural and/or biophysical approaches, especially nuclear magnetic resonance (NMR)-related techniques. The newly developed Magnetic Resonance Research Center at ASU **(website: http://nmr.asu.edu/)** provides a state-of-the-art NMR facility, including a new 800 megahertz spectrometer. Further information about the position can be obtained from the **Chair of the Search Committee, Jeffery Yarger (e-mail: jeff.yarger@asu.edu).** Applicants should send their curriculum vitae, a summary of future research plans, and arrange to have three letters of reference mailed to:

Prof. Jeffery L Yarger, Chair
Nuclear Magnetic Resonance and Biophysical Chemistry Search Committee
Department of Chemistry and Biochemistry
Arizona State University
P.O. Box 871604
Tempe, AZ 85287-1604

or e-mail material to **e-mail: mrrc@asu.edu.**

Review of applications begins December 12, 2007; if not filled, every two weeks thereafter until search is closed. *Background check is required for employment. ASU is an Equal Opportunity/Affirmative Action Employer and is committed to excellence through diversity.*

ENDOWED CHAIR in the BIOCHEMICAL SCIENCES
Department of Chemistry
University of Missouri-Rolla

Distinguished scientists are encouraged to apply for the **RICHARD K. VITEK/FOUNDATION for CHEMICAL RESEARCH (FCR) ENDOWED CHAIR in BIOCHEMISTRY,** including the areas of bioorganic, bioinorganic, biophysical, or biomaterials chemistry at the University of Missouri-Rolla (UMR). The new position carries a very generous endowment, which can be used to support the research of the Chair. The Richard K. Vitek/FCR Endowed Chair in Biochemistry will provide important leadership for UMR's targeted growth in the biosciences. This is an exciting time to join the Chemistry Department at UMR. A new Biosciences Building has been established by the state of Missouri as the next capital improvement project for higher education. Effective January 1, 2008, UMR will become the Missouri University of Science and Technology (MS and T).

The successful candidate should have a Doctorate in chemistry, biochemistry, or a related field, have an outstanding international reputation and publication record, and have a substantial record of extramural funding. This search has been extended, and review of applications will resume on December 15, 2007, and continue until the position is filled. For further information, we encourage you to visit our **website: http://chem.umr.edu** or contact **Prof. Jay A. Switzer** at **e-mail: jswitzer@umr.edu.**

Please submit curriculum vitae and short summaries of past research accomplishments and future research directions to: **Human Resource Services, Reference Number: 00033199, University of Missouri-Rolla, 1870 Miner Circle, Rolla, MO 65409-1050.**

UMR is an Affirmative Action/Equal Opportunity Employer. Women, minorities, and persons with disabilities are encouraged to apply.

POSITIONS OPEN

ASSOCIATE DIRECTOR, FRIDAY HARBOR LABORATORIES
The University of Washington

The University of Washington seeks applications for a tenure-track faculty position at the **ASSOCIATE PROFESSOR** rank, with concurrent appointment as Resident Associate Director of the University's Friday Harbor Laboratories (FHL). FHL, located on San Juan Island 90 miles north of Seattle, provides research and teaching facilities, and housing for over 250 full-time and temporary residents (see **website: http://depts.washington.edu/fhl/**). Research at FHL includes most areas of marine science, basic biological sciences focused on marine and aquatic organisms, and ecology of the terrestrial biota of the region. For this position, area of research can be in any marine science discipline (e.g. biology, fisheries, oceanography, engineering); the faculty appointment can be in any appropriate department at the University of Washington's Seattle campus. A record of outstanding achievement, a commitment to undergraduate and graduate teaching, public outreach and administrative experience, and a promising externally funded research program are important considerations.

Appointment at the Associate Professor rank is anticipated. In exceptional circumstances, appointment at the advanced **ASSISTANT PROFESSOR** level may be considered. Appointment at the **FULL PROFESSOR** rank may also be considered for outstanding candidates who have demonstrated a commitment to mentoring underrepresented students in the sciences. Applicants should have the Ph.D. degree by the date of appointment.

Applications, including a cover letter, curriculum vitae, statements of administrative, research and teaching interests, and names of at least three references should be provided. Please apply online at **website: http://fhl.washington.edu/jobsearch** (for information, contact: **Kenneth P. Sebens, e-mail: sebens@u.washington.edu).** Priority will be given to applications received before December 15, 2007.

University of Washington faculty engage in research, teaching, and service. *The University of Washington is building a culturally diverse faculty and strongly encourages applications from women, minorities, individuals with disabilities, and covered veterans. The University is an Affirmative Action/Equal Opportunity Employer.*

ASSISTANT PROFESSOR, tenure track. The Department of Zoology at Oklahoma State University **(website: http://zoology.okstate.edu)** invites applications for three positions: (1) **EVOLUTIONARY BIOLOGIST** specializing in environmental genomics, quantitative genetics, evolutionary development, sex-determining systems, or coevolution; (2) **DISEASE ECOLOGIST** specializing in infectious disease dynamics, transmission, and/or resistance from theoretical and experimental perspectives; and (3) **INTEGRATIVE BIOLOGIST** specializing in ecosystem ecology (emphasizing nutrient cycling, structure and function, or biocomplexity) or behavioral biology (emphasizing neural mechanisms, physiological ecology, or conservation). Candidates are expected to have a Ph.D. and postdoctoral research experience; responsibilities will include establishing vigorous, extramurally funded research programs, successfully mentoring M.S. and Ph.D. students, and effectively teaching at the undergraduate and graduate level. Candidates should submit (preferably by e-mail) a letter of application indicating the position to which they are applying, curriculum vitae, statements of research and teaching interests, three letters of recommendation (sent directly by the candidates' references), and up to three publications to: **Dr. Matt Lovern (e-mail: matt.lovern@okstate.edu), Chair, Faculty Search Committee, Department of Zoology, Oklahoma State University, 430 Life Sciences West, Stillwater, OK 74078.** Application review will begin 15 December 2007, with employment slated for August 2008; hiring is subject to available funding. *Women and minorities are strongly encouraged to apply. Oklahoma State University is an Equal Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

PROTEOMICS FACULTY POSITION ASSOCIATE/FULL PROFESSOR
University of Illinois at Chicago

The University of Illinois at Chicago (UIC) invites applications for a tenured faculty position starting in spring 2008.

We are searching for an outstanding candidate who is a leader in using proteomics and systems biology approaches as tools to study human disease. We particularly encourage applications from researchers using mass spectrometry who can take advantage of our new Fourier Transform among our suite of other state-of-the art mass spectrometers at UIC.

The successful candidate will join a cadre of faculty in the Chicago area who participate in the Chicago Biomedical Consortium (CBC). The CBC is a new initiative, generously funded through the Searle Funds in the Chicago Community Trust. The mission of the CBC is to stimulate and nurture major research collaborations relating to systems biology among scientists at Northwestern University, the University of Chicago, and the University of Illinois at Chicago.

The candidate must possess strong interpersonal, communication, project management skills, and strong records of collaboration across disciplines, and scholarship commensurate with the rank of Associate or Full Professor. Doctorate required. Compensation is competitive and commensurate with experience.

The search is being conducted jointly by the Colleges of Liberal Arts and Sciences, Pharmacy, and Medicine. For fullest consideration please send curriculum vitae, a brief summary of research plans, and three references by December 31, 2007, to **e-mail: cbc@uic.edu** or via **fax: 312-413-0238.** Materials also may be mailed to:

K. Colley, Chair of the Search (CBC-1)
Attn: E. Sauvee
Office of the Vice Chancellor for Research
The University of Illinois at Chicago, MC 672
310 Administrative Office Building
1737 West Polk Street
Chicago, IL 60612-7727

The position will remain open until filled. *UIC is an Affirmative Action/Equal Opportunity Employer*

VIROLOGY SEARCH: TENURE-TRACK ASSISTANT PROFESSOR
Department of Microbiology
University of Washington

The Department of Microbiology at the University of Washington in Seattle is conducting a search for an Assistant Professor in the field of animal virology. We are looking for an innovative investigator who will develop an independent research program utilizing modern technologies to study viral pathogenesis, virus-host interactions, viral immunology, or systems virology. The position is a 12-month, full-time, tenure-track position in the School of Medicine. In addition to research, the new faculty member will support the Department's teaching mission including teaching at the undergraduate or graduate level. University of Washington faculty engage in teaching, research, and service.

Salary and benefits are competitive and will be commensurate with the qualifications and experience of the applicant. Applicants with a Ph.D. and a minimum of two years of postdoctoral experience and a strong publication record should send their curriculum vitae and a one or two-page statement of research interests, and arrange to have three letters of reference sent to: **Chair, Virology Search Committee, Department of Microbiology, P.O. Box 357242, University of Washington, 1705 N.E. Pacific Street, Seattle, WA 98195.** Application deadline: January 10, 2008.

The University of Washington is an Affirmative Action, Equal Opportunity Employer and is building a culturally diverse faculty. Applications from female and minority candidates are encouraged.

POSITIONS OPEN

POSTDOCTORAL RESEARCHER to participate in National Science Foundation-funded study in comparative grass regulomics (maize, sorghum, sugarcane, and rice). **(Website: http://www.utoledo.edu/as/bio/research/gray.html.)** The Associate will overexpress maize transcription factors and conduct chromatin-immunoprecipitation experiments to define gene regulatory networks. Ph.D. in biology required. Review of applications will begin immediately. Submit resume and three references to: **Dr. John Gray, Biology Science Department, Mailstop 601, The University of Toledo, 2801 W. Bancroft Street, Toledo, OH 43606-3390, fax: 419-530-1538,** or **e-mail: jgray5@utnet.utoledo.edu** (reference job #T99121 in subject line).

MARKETPLACE